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Vol. 1

Canadian Journal of Botany

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VOLUME 39 OCTOBER 1961 NUMBER 6

A NEW HELICOSTYLUM FROM INDIAN SOILS[1]

J. N. RAI, J. P. TEWARI, AND K. G. MUKERJI

Abstract

A species of *Helicostylum* Corda isolated from samples of soil (pH 6.0–8.0) collected from Lucknow (U.P.), India, was found to be different from all the known species of this genus and has been described as *Helicostylum lucknowense* Rai, Tewari and Mukerji n. sp.

Introduction

The genus *Helicostylum* was instituted by Corda (1) in 1842 for mucoraceous fungi resembling *Thamnidium elegans* Link but with circinate pedicels and no dichotomous branching, with *H. elegans* Corda as the type species. Saccardo (6) recorded five valid and one doubtful species of this genus. Zycha (7) recorded *H. elegans* as a species of *Chaetostylum* van Tieghem et le Monnier and listed four species under the genus *Helicostylum*. Naumov (5), while recognizing the validity of Corda's type species, listed some eight other species under this genus. Hesseltine (3) in a general study of the genera of Mucorales reported five or six species of this genus and treated *Haynaldia* Schulzer v. Muggenburg and *Thamnidium* of the same authors as synonymous with *Helicostylum*. Fitzpatrick (2) reduced its status to a subgenus of *Thamnidium* Link but recent workers like Zycha (7), Naumov (5), Hesseltine (3), and Lythgoe (4) have emphasized the validity of Corda's genus. While the presentation of a detailed account of the taxonomic treatments of *Helicostylum* and allied genera is beyond the scope of this paper, the above may suffice to indicate the confusion that exists as to the number of valid species of this genus and as to its taxonomic position.

Lythgoe (4) has discussed the three taxonomic criteria used for separating *Helicostylum* and *Chaetostylum*, i.e. type of branching, form of the pedicels and columella of the sporangiole. The form described here bears good rhizoids and stolons. There seems to be a general paucity of descriptions of these structures in the taxonomic accounts of this genus. According to Hesseltine (personal communication) "there are species in this family (i.e. Thamnidiaceae) which do have rhizoids and stolons but this character has never been

[1]Manuscript received February 27, 1961.
Contribution from the Microbiology Laboratory, Botany Department, University of Lucknow, Lucknow, India.

[The date of issue of the previous number of Can. J. Botany (39, 997–1280) will be given in the next issue.]

given much importance in the classification of this family." It is of interest to note that a character which has been given much importance in one family (i.e. Mucoraceae) should not be considered at all in another sister family (i.e. Thamnidiaceae).

The present publication deals with a fungus isolated from soil and identified as a species of *Helicostylum* because of the presence of pyriform sporangiola borne on circinate pedicels, the presence of terminal *Mucor*-like sporangia, and the lack of dichotomous branching. A further study revealed it to be distinct from all the hitherto-reported species of this genus. It has thus been named *Helicostylum lucknowense* Rai, Tewari and Mukerji n. sp., the specific epithet being derived from the place of origin.

Description

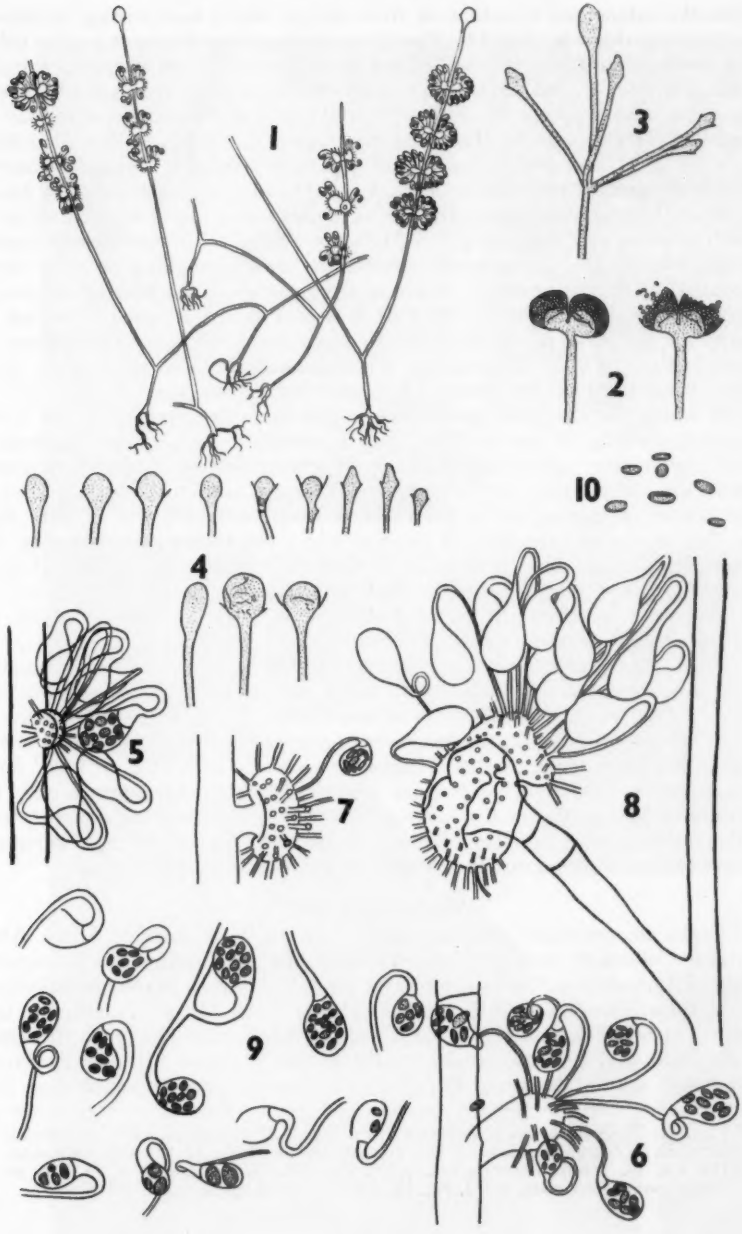
Helicostylum lucknowense spec. nov.

Coloniae albae, circulares, marginibus aliquantum dissectis, ca, 1.5–2.5 cm altae, diametrum attingentes ca. 9.8 centimetrorum post dies 6 incubation is sub $30 \pm 1°$ C in obscuritate in agaro Czapek-Dox + 0.5% fermenti extracto; versa facies incolora. Sporangiophori erecti, saepe furcati, ut plurimum 0.8–5.0 mm longi, 5.0–19.2 μ lati, supportantes sporangium terminale et 1–8 nodos sporangiales et surgentes vel ex mycelio submerso vel ex stolonibus ferentibus rhizoideas ramosas et septatas. Sporangia terminalia Mucori similia, 29.6–80.0 $\mu \times$ 22.2–59.2 μ, columellis varie formatis et magnit. 8.4–34.0 $\mu \times$ 14.8–30.4 μ, parietibus diffluentibus collulo distincto relicto, multispora. Singuli nodi sporangiales constant una vel pluribus tumescentiis quae supportant plurimos sporangiolophoros circinnatos 35.2–64.0 $\mu \times$ 1.0–2.4 μ, quorum singuli sua vice supportant unum sporangiolum terminale. Sporangiola pyriformia, parietibus persistentibus, saepe supportantia columellas parvas, caduca, 2–16 spora, 8.0–16.0 $\mu \times$ 7.0–11.2 μ. Sporae ovales vel virgae similes, hyalinae, laeves, 3.2–7.0 $\mu \times$ 1.6–3.2 μ. Zygosporae haud visae.

Fungus extractus e solo (pH 6.0–8.0) lecto mensibus octobri ad decembrem, 1959 e regione Kukrail et Daligang, ad Lucknow (U.P.) in India; cultura typica posita in ARS Cult. Collect. Invest., Fermentation Laboratory, U.S. Dept. Agric., Peoria, Illinois, sub numero A–10, 034.

Colonies on Czapek-Dox agar + 0.5% yeast extract at $30 \pm 1°$ C in dark at 6 days of age white, circular with a slightly dissected margin, about 9.8 cm in diameter, reaching lid of the petri dish and turning light pale-yellow after about 2 weeks' incubation. Reverse agar uncolored and colony reverse zonate in young age. Sporangiophores (Figs. 1, 11) smooth, erect, frequently branched, generally 0.8–5.0 mm in length and 5.0–19.2 μ in width, bearing a terminal sporangium and 1–8 sporangiolar nodes and arising either

FIGS. 1–10. *Helicostylum lucknowense* n. sp. Fig. 1. Complete plants, ×50. Fig. 2. Terminal portion of a sporangiophore showing two dehisced and undehisced sporangia borne on the same sporangiophore; the spores are still immature; ×600. Fig. 3. Terminal portion of a sporangiophore showing five dehisced sporangia borne on the same sporangiophore, ×600. Fig. 4. Columellae, ×600. Figs. 5–8. Portions of sporangiophores showing sporangiolar nodes, ×600. Fig. 9. Dehisced and undehisced sporangiola, ×600. Fig. 10. Spores, ×600.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from the submerged mycelium or from stolons which bear strong, branched, and septate rhizoids (Fig. 12). The sporangiophore for the most part is colorless and comparatively thin-walled but at the base and from where the rhizoids arise it is thick-walled and brown in color. Rarely a single sporangiophore may bear two or more sporangia (Figs. 2, 3) and rarely the sporangiophore may be terminated by a spine also. Terminal sporangia (Fig. 13) *Mucor*-like, 29.6–80.0 $\mu \times 22.2$–$59.2\ \mu$, wall diffluent and generally leaving a distinct collarette and multisporous. Columellae (Figs. 4, 14, 15) ranging in shape from hemispherical to dome-shaped and conical, at times bearing one or a few small teeth-like processes and measuring 8.4–$34.0\ \mu \times 14.8$–$30.4\ \mu$. Sporangiolar nodes (Figs. 5–8, 16, 17), either borne directly on the sporangiophore or at times situated on short branches, consist of irregular swellings bearing numerous circinate sporangiole-phores 35.2–$64.0\ \mu \times 1.0$–$2.4\ \mu$, each of which in turn bears a terminal sporangiole. In some cases the sporangiole-phore is branched at the base. Sporangiolar nodes after shedding of sporangiola show short basal parts of the sporangiole-phores and small round perforations in cases where the complete sporangiole-phores have dropped out (Figs. 5–8). Sporangiola (Fig. 9) are pyriform, with a persistent wall, frequently bearing small columellae, caducous and break off with a portion of the sporangiole-phore, generally from 2- to 16-spored and measure 8.0–$16.0\ \mu \times 7.0$–$11.2\ \mu$. Dehiscence of sporangiola is by means of undetermined pores or slits that develop on the sporangiole wall through which the spores escape leaving the empty sporangiola. Spores (Fig. 10) of both the terminal sporangia and sporangiola are oval to rod-shaped, hyaline, smooth, and 3.2–$7.0\ \mu \times 1.6$–$3.2\ \mu$. Although cultures from different isolations have been extensively mated, no zygospore production could be observed.

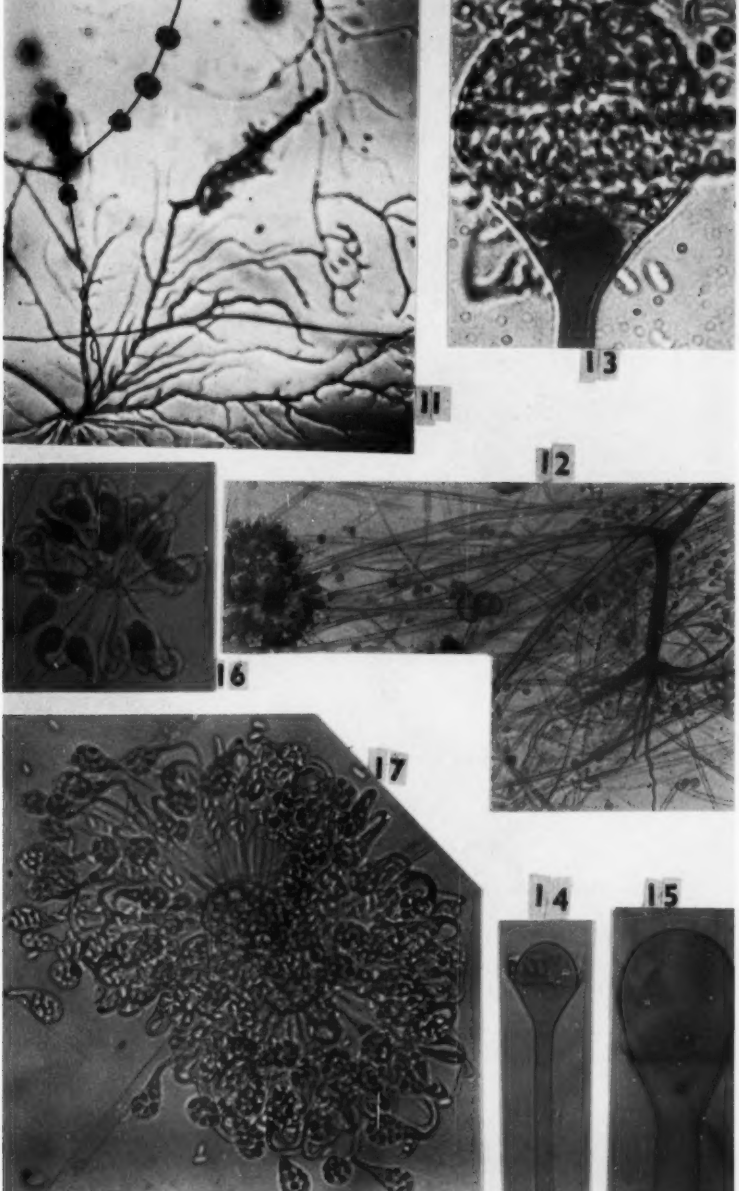
Helicostylum lucknowense n. sp. was isolated using the soil dilution method from samples of soil collected from a depth of 6 inches from October to December, 1959, from Kukrail (pH 6.0) and Daligang (pH 8.0) areas, Lucknow (U.P.), India. In Kukrail, the locality was a grassland near the Kukrail River with a few palm trees, and in Daligang it consisted of a plot of 'Usar' land (alkali) with patches of poor grass growth. In this connection it may be mentioned that only very few species of the genus *Helicostylum*, and they too rather infrequently, have been recorded from soil. Most of the reports record their isolation from animal faeces or from decomposing animal tissue.

Acknowledgments

Thanks are gratefully acknowledged to Dr. C. W. Hesseltine, Head, ARS Culture Collection Investigations, Fermentation Laboratory, U. S. Department of Agriculture, Peoria, Illinois, for his valuable help in the identification of the form. The junior authors (J. P. T. and K. G. M.) are indebted to the Council of Scientific and Industrial Research, New Delhi, and to the Ministry of Scientific and Cultural Affairs, India, for the grant of a Junior Research Fellowship and a Research Training Scholarship, respectively, during the

FIGS. 11–17. *Helicostylum lucknowense* n. sp. Fig. 11. Sporangiophores with rhizoidal system; the photograph was taken from a living culture; ×30. Fig. 12. Rhizoids and stolons, ×114. Fig. 13. Terminal sporangium, ×999. Figs. 14, 15. Columellae, ×513. Fig. 16. A young sporangiolar node, ×513. Fig. 17. A mature sporangiolar node, ×419.

PLATE I



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tenure of which this work has been done. Thanks are also due to Rev. Fr. Prof. Dr. H. Santapau of St. Xavier's College, Bombay, for translating the specific diagnosis into Latin.

Addendum

Since this paper has been sent to press, Dr. Richard K. Benjamin of Rancho Santa Ana Botanic Garden, Claremont, California, has informed us (personal communication) that the Indian strain of *Helicostylum lucknowense* Rai, Tewari and Mukerji n. sp. is compatible with his four North American strains (three from California and one from Mexico) and readily produces zygospores when crossed.

The type culture of this form has also been deposited in Northern Utilization Research and Development Division, U. S. Department of Agriculture, under reference No. NRRL 2892, and in the Commonwealth Mycological Institute, Kew, England, the reference number being not yet known.

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*Not seen in original.

FIELD GRAFTING WITH HEALTHY AND NEEDLE-BLIGHTED EASTERN WHITE PINE TREES AND THE EXPRESSION OF DISEASE SYMPTOMS[1]

S. N. LINZON[2]

Abstract

Healthy and needle-blighted eastern white pine trees (*Pinus strobus* L.) were intergrafted in the forest using bottle and cleft crown graft techniques. The grafts were made in four combinations of diseased and healthy scions and stock branches and at three different stages of development of the graft components. High percentages of successful grafts were obtained when the partner combinations included healthy scions and when the grafting was done in early May with active top growth just beginning in both scions and stock. Fewer grafts survived when diseased scions were employed and when the field grafting was carried out with dormant-collected scions or with scions and stock both possessing advanced new growth.

Each grafted partner retained its original identity. Needle-blight symptoms appeared simultaneously on diseased scions and their mother trees, while the healthy stock trees remained unaffected. Conversely, healthy scions and their mother trees were unaffected while the diseased stock trees displayed the needle-blight symptoms on their foliage. No disease symptoms were transmitted by stem grafting. A search revealed that perennially needle-blighted trees were joined to neighboring healthy trees by natural root grafts, and that there was no visible communication of the disease symptoms. Additional evidence has been presented to show that needle blight is nonparasitic in etiology and that susceptibility to needle breakdown is inherent in the individual.

Introduction

Needle blight of eastern white pine is characterized by an orange-red discoloration of the distal portions of current-year needles. It was recently discovered (3) that contrary to being a needle dieback, the injury initiates in semimature leaf tissues and then spreads distally to more-mature tissues. Based on data with respect to the nature and occurrence of the disease, the hypothesis was set forth that the response of trees in exhibiting the foliar symptoms when subjected to unfavorable conditions was a heritable trait (3).

Personal communication with Dr. C. C. Heimburger and Mr. B. W. Dance, Maple, Ontario, and correspondence with Dr. A. J. Riker, Madison, Wisconsin, have revealed that scions from needle-blighted trees which had been grafted to healthy rootstock were reaffected in later years while the healthy stock remained free of disease symptoms. The reverse grafts were not attempted by these workers.

In this study several series of healthy and needle-blighted trees were intergrafted in various combinations. These grafts were made in the field at the Petawawa Forest Experiment Station, Chalk River, Ontario, in 1959.

Materials and Methods

The grafting work was conducted in two white pine plantations, aged 11 and 14 years. Two types of grafts were undertaken, bottle and cleft crown.

[1]Manuscript received June 5, 1961.
Contribution No. 772, Forest Entomology and Pathology Branch, Canada Department of Forestry, Ottawa, Canada.
[2]Forest Pathology Laboratory, Maple, Ontario.

TABLE I

Schedule of field grafting with eastern white pine

Date, 1959	Series A and Series B	Series C
	Bottle graft technique	
May 6–7	Scions united to stock branches	
May 26–27		Scions united to stock branches (Figs. 1–3)
June 8	First cut on stock branches above grafts, removing one-half of current growth	
June 15	Second cut on stock branches removing remainder of current growth	First cut on stock branches above grafts removing one-half of current growth
June 22		Second cut on stock branches, removing remainder of current growth
June 30	Third cut on stock branches above grafts, removing one-half of 1-year-old growth (Fig. 4)	
July 7–8	Removal of constricting rubber grafting bands from grafted areas (Fig. 5), and union tied loosely with tape and covered with aluminum foil	
Aug. 5	Final cut on stock branches above grafts, and cut ends waxed	
Aug. 14	Final cut on scions below grafts, vials removed, and cut ends waxed (Fig. 6)	
	Cleft crown graft technique	
May 8	Scions united to stock branches (Figs. 7–9)	
May 28		Scions united to stock branches
July 8	Removal of constricting rubber grafting bands from grafted areas and replacement of plastic and paper bags over grafts	
Sept. 1	Final removal of plastic and paper bags (Fig. 10)	

Intergrafts were made between trees, with histories either of disease recurrence or of freedom from blighting, located in the same plantation. Four combinations of intergrafts were made: (1) healthy scions × healthy stock branches (H×H); (2) healthy scions × needle-blighted stock branches (H×NB); (3) needle-blighted scions × healthy stock branches (NB×H); and (4) needle-blighted scions × needle-blighted stock branches (NB×NB). These four combinations were repeated at three different stages of development of scions and stock. These stages were as follows:

Series A.—Both scions and stock with buds just breaking and active top growth just starting (early May at Chalk River). Scions were detached from mother trees and attached to branches of stock trees on the same day.

FIGS. 1 to 6. Bottle graft technique. Fig. 1. Cuts on scion (1-year-old shoot) and stock branch (2-year-old growth) being matched. Fig. 2. After the cambia were aligned, the scion was bound securely to stock branch with a rubber grafting band. Fig. 3. The union was sealed with grafting wax. The base of the scion was clipped and placed in a vial of water. Fig. 4. The stock branches were cut back gradually. Note union covered with aluminum foil. Fig. 5. Rubber grafting band removed to prevent strangulation of union. Grafted area then tied loosely with tape. Fig. 6. Appearance of graft 1 year after final cuts have been made on stock branch and base of scion. Vial has been removed, and the cut ends have been sealed with grafting wax. Periodically during the grafting procedure the grafted scion was sprayed with pesticides to combat fungi and insects.

PLATE I

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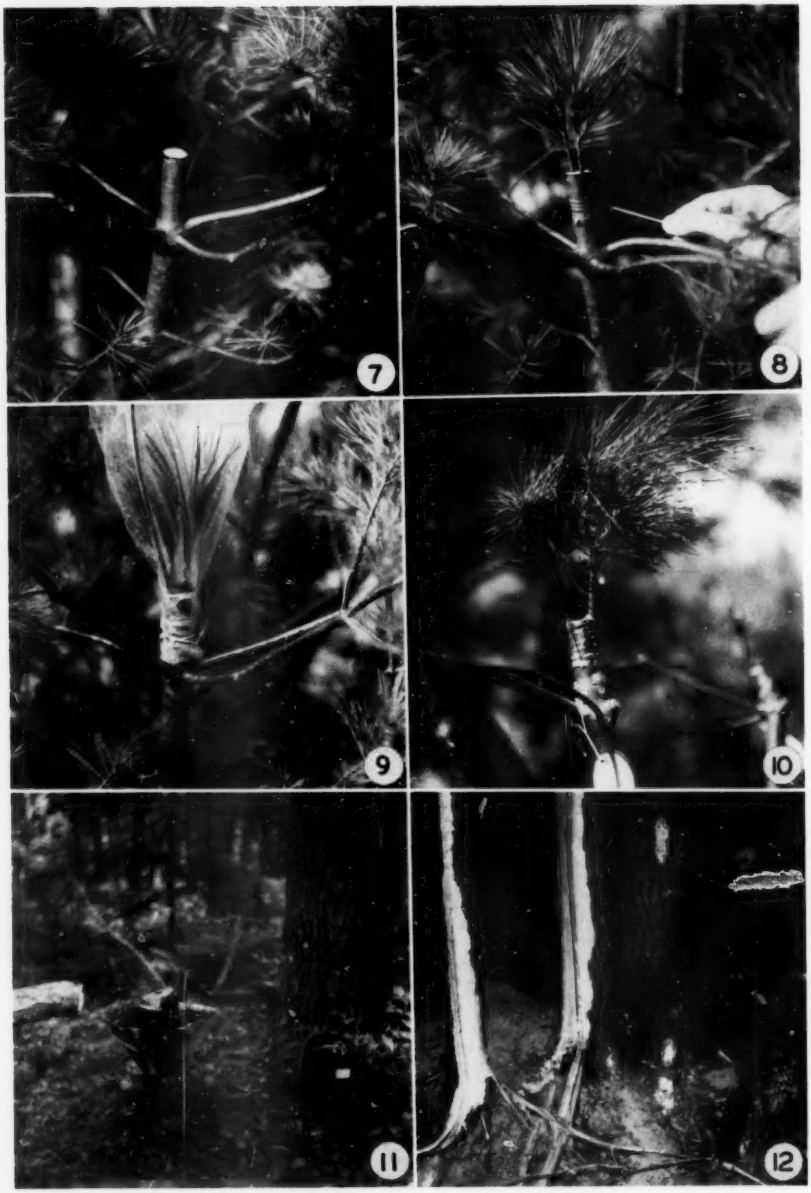

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Series B.—Scions collected from mother trees when dormant (March) and kept in deep freeze until grafted on to stock branches in early May when active top growth was just starting.

Series C.—Both scions and stock with advanced succulent current growth (late May). Scions were detached from mother trees and attached to branches of stock trees on the same day.

Excellent instruction in making bottle grafts with slash pine is given by Mergen and Rossoll (4). In white pine the timing of the various steps was different. The schedule used in this study for making both bottle and cleft crown grafts is shown in Table I. The captions for Figs. 1 to 10 describe the techniques used.

Four scions were attached to each stock tree. A total of 64 bottle grafts and 16 cleft crown grafts were made in May, 1959. Graft survival tallies were taken at the end of the 1959 growing season, in the spring of 1960, and again at the end of the growing season in 1960. During the summer of 1960 the grafted scions, the stock trees, and the mother trees were periodically examined for needle growth, needle color, and the appearance or nonappearance of the foliar symptoms of needle blight.

Results and Discussion

Table II outlines the survival tally for both bottle and cleft crown grafts. At the time of removal of the rubber binding in early July, 1959, all 80 grafts appeared united with no failures observed. However, 35 grafts died during the summer of 1959, of which 24 possessed needle-blighted scions. Of 14 grafts lost over the winter of 1959–60, eight were rabbit-browsed. No grafts died during the summer of 1960.

The best over-all rate of graft survival occurred in Series A, with 75% of both kinds of grafts still living after two growing seasons. In Series B, the dormant-collected scions united well with the stock branches, but scant new growth was made by the grafts, and mortality was high during the first summer. It was unfortunate that of eight grafts lost through rabbit-browsing over the winter of 1959–60, six were in Series B. Fifty per cent bottle graft mortality and 75% cleft crown graft mortality occurred in Series C during the first summer. Apparently the shock of detaching scions possessing new stump growth was difficult to overcome.

FIGS. 7 to 10. Cleft crown graft technique. Fig. 7. Decapitated branch on stock tree prior to being split. Fig. 8. A needle-blighted scion (on left) and a healthy scion inserted side by side into cleft made in stock branch. After the cambia were aligned, the unions were bound firmly with a rubber grafting band and then sealed with grafting wax. Fig. 9. A wire hoop was fitted over grafts and tied to stock branch. A moistened polyethylene plastic bag was placed over the hoop, and this was followed by a paper bag cut at two corners for ventilation. Fig. 10. The constricting rubber grafting band was removed in early July. The plastic and paper bags were removed in early September. New growth was made by the grafts under cover during the summer of 1959. Periodically during the summer the covers were lifted and the grafts were sprayed with pesticides to combat fungi and insects. The photograph shows the appearance of the cleft crown grafts 1 year after unions occurred.

FIGS. 11 and 12. Natural root grafting. Fig. 11. Needle-blighted tree was felled and stump was injected with a stain solution. Fig. 12. Needle-blighted tree joined to neighboring healthy trees by natural root grafts.

TABLE II

Survival of field grafts made between healthy and needle-blighted trees

Graft combination	No. of grafts	July 8, 1959		Sept. 16, 1959		May 25, 1960		Aug. 31, 1960	
		No.	%	No.	%	No.	%	No.	%
Bottle graft survival									
Series A									
H×H	4	4		4		4		4	
H×NB	4	4		4		4		4	
NB×H	4	4		4		3		3	
NB×NB	4	4		2		1*		1	
Total or average	16	16	100.0	14	87.5	12	75.0	12	75.0
Series B									
H×H	4	4		2		0*		0	
H×NB	4	4		4		0*		0	
NB×H	4	4		1		1		1	
NB×NB	4	4		0		0		0	
Total or average	16	16	100.0	7	43.8	1*	6.3	1	6.3
Series C									
H×H	8	8		5		4		4	
H×NB	8	8		7		6*		6	
NB×H	8	8		2		2		2	
NB×NB	8	8		2		0		0	
Total or average	32	32	100.0	16	50.0	12	37.5	12	37.5
Bottle grafts, total or average	64	64	100.0	37	57.8	25	39.1	25	39.1
Cleft crown graft survival									
Series A									
H+NB×H	2	2		2		2		2	
H+NB×NB	2	2		2		1		1†	
Total or average	4	4	100.0	4	100.0	3	75.0	3	75.0
Series B									
H+NB×H	2	2		2		1		1†	
H+NB×NB	2	2		0		0		0	
Total or average	4	4	100.0	2	50.0	1	25.0	1	25.0
Series C									
H+NB×H	4	4		1		1		1†	
H+NB×NB	4	4		1		1		1†	
Total or average	8	8	100.0	2	25.0	2	25.0	2	25.0
Cleft crown grafts, total or average	16	16	100.0	8	50.0	6	37.5	6	37.5
All grafts, total or average	80	80	100.0	45	56.3	31	38.8	31	38.8

*Graft loss due to rabbit-browsing.
†Needle-blighted scion survived.

The success of grafts was also influenced by the combination of grafted partners. The numbers of surviving grafts declined when the combinations included diseased scions. This would be expected, since needle-blighted trees, in addition to having their foliage injured, would lack food reserves because of their smaller foliage and shoot growth in comparison with that made by neighboring healthy trees (5).

In Series A, 100% survival of bottle and cleft crown grafts occurred when the partners were both of healthy origin, or when healthy scions were bottle-grafted to diseased trees. This indicates that high percentages of successful grafts may be expected when scions from healthy trees are united to understock in the field, at the time when active top growth is just beginning for both partners.

The occurrence or absence of needle-blight symptoms on new foliage of grafted scions, stock trees, and mother trees was closely observed during the summer of 1960. The development of foliar symptoms on the grafts during 1959 was not considered, since it was a year of graft establishment.

In addition to finding needle-blighted scions reaffected in 1960 while the healthy understock remained unaffected, healthy scions were found to be unaffected on needle-blighted understock which displayed the foliar symptoms in 1960. In every case, the development of the new foliage on the scions, whether from diseased or healthy origin, was similar to that occurring on the mother trees in the same local area. These similarities embraced needle growth, needle color, and appearance or nonappearance of needle-blight symptoms.

Diseased scions exhibited small, discolored foliage when compared with the foliage on their healthy understocks. Foliage on healthy scions, in addition to being unaffected, was much larger than the injured foliage on diseased stock trees. Not only did typical needle-blight symptoms develop on the 1960 foliage of both scions and their diseased mother trees, but in one instance the foliage of a mother tree displayed excessive chlorotic spotting, and this type of injury appeared similarly on the foliage of scions which had been derived from this tree. In cleft crown grafts the needle-blighted scion was reaffected while the healthy insert beside it was unaffected.

The most remarkable phenomenon observed was that needle-blight symptoms occurred simultaneously on foliage of diseased mother trees and the scions which were removed from them and grafted on to other trees in the same plantation. The symptoms initiated in needle tissue of the same stage of maturation, and the rate of distal spread of foliar necrosis was comparable in the new foliage of scions and their diseased mother trees. The healthy stock trees were unaffected.

Preliminary to this study a small number of Series C bottle grafts, in the four combinations of diseased and healthy partners, were made in the field in late May, 1958, at Chalk River. During the summers of both 1959 and 1960 observations revealed similar results to those found in 1960 for the 1959 Series C bottle grafts.

There was no transmission of disease symptoms from needle-blighted scions to healthy stock trees, or from diseased stock trees to healthy scions. Faull (2) also found the disease to be noncommunicable in both stem-grafting and

juice-inoculation experiments. In some plant diseases caused by viruses the organism is transmitted by root grafts. Intraspecific, natural root grafting between white pines in the forest is very common (1). Since needle-blighted and healthy trees are often found growing side by side, an investigation was conducted to see whether or not a perennially blighted tree was united by natural root grafts to neighboring trees which had never been observed to exhibit the symptoms of needle blight.

A diseased tree was felled. An aluminum pan in the shape of a cone-frustum was affixed to the stump and a 0.3% aqueous solution of acid fuchsin was added to the pan (Fig. 11). The stump was girdle-incised below the level of the solution in the pan. After 2 gallons of solution had been absorbed by the stump the injection was stopped. The bark was then lifted from the stems of neighboring healthy trees, and the stain solution was seen to have ascended in their trunks. Careful digging about the bases of these trees revealed that the diseased tree was united to two healthy trees by grafted small roots. In addition, one of these healthy trees was passing the stain solution on to another adjacent healthy tree by a root graft (Fig. 12). Evidently there is a natural underground complex of root-grafted healthy and needle-blighted white pine trees in the forest, with no communication of disease symptoms occurring. This situation, however, may complicate the control of needle blight, since poisons used to eradicate diseased white pine seed trees may travel through the roots to neighboring healthy trees.

With the knowledge that disease symptoms cannot be communicated, and that in grafting trials each partner member retained its original identity, additional evidence has been provided to support the hypothesis that the cellular make-up of needle-blighted trees which makes them susceptible to needle breakdown is an inherited trait.

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BOTANICAL STUDIES ON MONT ST. HILAIRE, ROUVILLE COUNTY, QUEBEC

GENERAL DESCRIPTION OF THE AREA AND A FLORISTIC SURVEY[1]

P. F. MAYCOCK

Abstract

The Monteregian Hills are a series of eight mountains, intrusive in nature, which form a distinct petrographical province in the St. Lawrence Lowlands of Southern Quebec. Mont St. Hilaire, one of the larger members of the group, was bequeathed to McGill University as a natural area for scientific research in 1959 by the late Brigadier A. Hamilton Gault. During a survey of the natural resources of the mountain throughout the summer of 1959, botanical investigations of the flora and vegetation were conducted. The present report is the first in a series that will present the results of these studies. It is concerned with the general characteristics of the mountain and the known existing flora.

Introduction

In the St. Lawrence River Valley of southern Quebec and adjacent Ontario an extensive low-lying plain was formed in postglacial times. The flat nature of this terrain is greatly accentuated by the Laurentian Highlands which occur to the northwest and the mountains of the Appalachian System lying to the southeast. This plain, hemmed in by these rugged elevated land masses, has been described as the St. Lawrence Lowlands. In the vicinity of Montreal and the region lying due east of the Island the surrounding lowlands are disrupted by a series of eight large hills, locally referred to as mountains. These hills are scattered on the plain between the Island of Montreal and the Town of Shefford in the Eastern Townships. One of the largest of these hills was first viewed by Europeans in the year 1535 when Cartier and his men sailed upriver to the Indian village of Hochelaga which was at the site of present-day Montreal. This hill, which is first sighted after leaving Lake St. Peter where the river veers east, is the mountain of St. Hilaire.

Mount Royal in the heart of the City of Montreal is the westernmost member of this group of hills which was recognized as a distinct petrographical province by Adams in 1903 (Adams 1903). Mount Royal (840 ft) was selected as the type for the group and its name has been ascribed to the province—The Monteregian Hills. The other members in order of their occurrence from west to east are Mont St. Bruno (672 ft), Mont St. Hilaire (1400 ft), Mount Johnson (958 ft), Rougemont (1368 ft), Mount Yamaska (1607 ft), Mount Shefford (1886 ft), and Mount Brome (1920 ft).

Much of the interest excited by the Monteregian Hills is related to their sudden topographic deviation from the lowlands and their markedly different geological origin, which has naturally been a subject of speculation and controversy. Of equally exciting interest are the striking vegetational differences, which are a reflection not only of the altitudinal changes which have

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given rise to climatic gradients, but also of the geological history to which the Hills have been subjected. Actually the contrasts between the biological, topographical, climatological, and geological characteristics of these hills and the surrounding lowlands combine to give rise to natural phenomena which merit the attention of scientists in these various disciplines and produce areas where fruitful scientific investigations may be carried out. Each area in itself can be considered as an extensive outdoor laboratory (Maycock 1959*c*).

The Montreal plain was settled in 1642 and extensive clearing of the forests for agriculture has largely left the Monteregians as stranded forest "oases". It is understandable then that they have served as centers for recreation and the enjoyment of the natural history of the St. Lawrence Valley. These activities are becoming increasingly limited, especially of recent years owing to the surging tide of population expansion and suburban infringement.

Many members of the group have been seriously disturbed by human settlement and encroachment. The lower terraces were early cleared extensively for apple cultivation. Suburban real estate developments now threaten the orchards as well as the remaining undisturbed sections of the mountains. Mont St. Hilaire, because of a peculiar history and the fact that a major portion of the summit has been in the hands of two land-owning families for many years, remains still relatively undisturbed and parts are still in the primeval state. Since 1914 the mountain has been the property of Brigadier A. Hamilton Gault and upon his death in 1959 he bequeathed the property to McGill University. The intention of his gift was to provide an opportunity for the pursuit of scientific studies, particularly in the natural sciences, without fear of subsequent disturbance or destruction and for the purpose of enlarging the teaching facilities in field subjects. The concept of an extensive natural area best describes his intended use. During the summer of 1959 a scientific survey was initiated, under the direction of the author, supported by research funds of the University, to ascertain the natural resources of the mountain, to initiate scientific projects, and to assist in planning the future utilization of Mont St. Hilaire in the scientific and teaching programs of the University.

The present report is the first in a series that will be concerned with the results of the scientific survey, particularly the botanical resources and the ecological relationships of the vegetational communities and component plants. It is intended to present the general features of the mountain and then to consider the flora as presently known.

Description of the Mountain

General Features

St. Hilaire Mountain is situated in the parish of St. Hilaire, County Rouville, approximately 20 miles northeast of Montreal. On clear days the mountain is distinctly visible from the top of Mount Royal. Historically the mountain was included in the seignory of Jacques de Chambly, received by royal charter around 1640. These holdings were later transferred by marriage to Jean B. Hertel de Rouville who later assumed the name of Chambly (Thwaites 1900). The single lake on the mountain is named Hertel in recognition of the second seigneur.

The lower slopes of the mountain begin to rise less than 1 mile east of the Richelieu River. Adjacent to these slopes and surrounding them on all sides are the municipalities of St. Hilaire, Mont St. Hilaire, St. Jean-Baptiste, and Ste. Madeleine. Otterburn Park, Beloeil, and McMasterville are nearby to the west.

The highest summit rises to an altitude of nearly 1400 ft, roughly 1300 ft above the surrounding lowlands. From an aerial view the mountain is circular in shape and occupies 9 square miles including the outer terraces. The main body, which is almost entirely included in the McGill holdings, comprises approximately one-half of this total area.

Topographic Features

From a distance Mont St. Hilaire appears as a solid dome-shaped structure but in adjacent situations a series of separate peaks receding into a central depression become evident. Actually the rock masses form a circular tower with an outer rampart enclosing an inner basin which is situated off-center and south of it. These topographic features are evident in Fig. 1. The inner slopes of this basin act as a watershed and the runoff waters collect in the basin to form Lac Hertel at an average elevation of 570 ft. The general topography of the mountain is best considered in relation to the lake.

The outer ramparts are broken by four small valleys which lead through cols to the interior basin. The troughs of the north, west, and east cols are collection areas and the three main streams which supply the lake originate in these situations. The only stream draining the lake leaves by the south col. The flow from this exit is controlled by an earthen and concrete dam, the original structure having been constructed previous to 1890.

The four cols referred to divide the mountain into four separate summits. Each of these summits may be broken by cols and ravines which give rise to more or less separate peaks. These peaks have been assigned names for reference purposes as have other topographic and geographic features. Many of these names are presented in Fig. 1. The main peaks are eight in number and are listed with their elevations in Table I.

TABLE I

The various peaks of Mont St. Hilaire and their elevations

Peaks	Elevation in feet
Sugar Loaf (Pain du Sucre)	1365
Rocky Mountain	1350
North Mountain	1275
East Mountain	1300
Lake Mountain	975
Small Mountain	875
Burned Mountain	1000
Hemlock Knolls	925

In general the tops of these main peaks, with the exceptions of the Sugar Loaf and Lake Mountain, are gently rounded and occupy several acres. The exceptions are abruptly rounded and possess little flat ground. These topographic features influence the vegetational characteristics of the summits

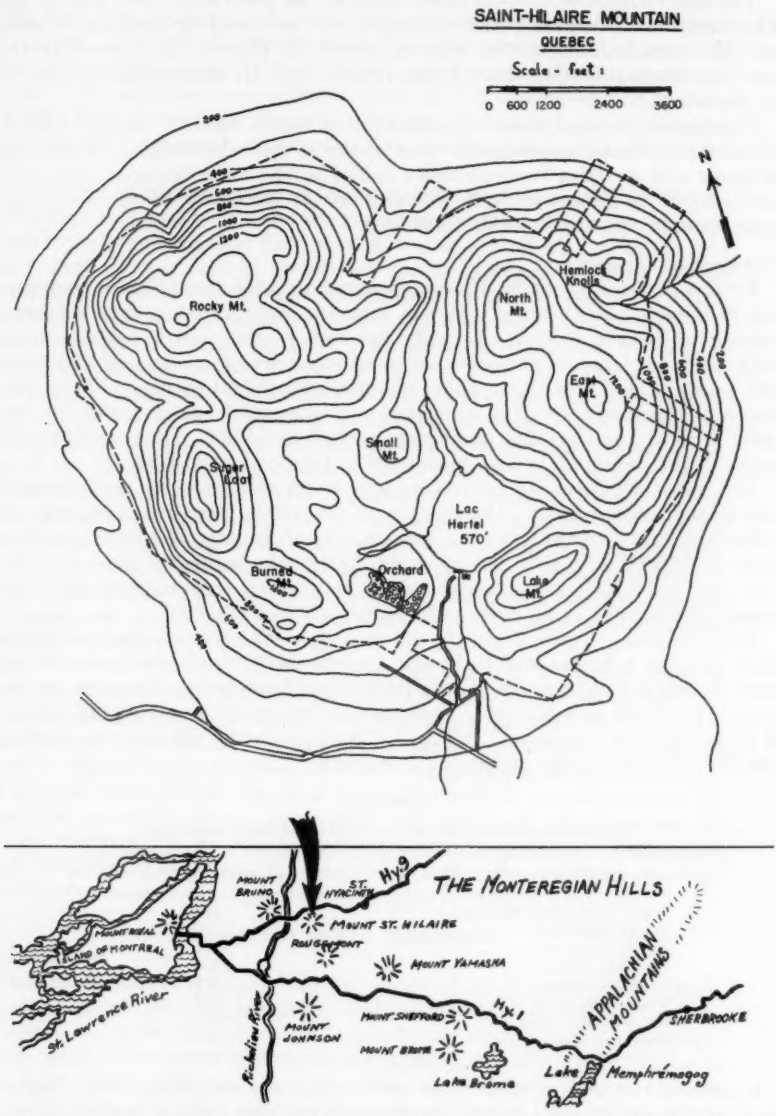

FIG. 1. Map showing the general topographic features of Mont St. Hilaire and presenting the geographical position in relation to the other members of the Monteregian Series of southern Quebec.

and thus the flat-topped peaks are covered with forest and afford no view of the surrounding terrain whereas the Sugar Loaf, a small promontory on the west side of North Mountain, and Hills 1 and 2 of Rocky Mountain are rocky and support open, low, shrubby plant communities.

The steepness of the slopes varies continuously over the area and this factor allied with other environmental features is also a major factor in determining vegetational composition. The slopes of the inner basin are considerably less drastic than those of the outer rim although variations to the contrary may be found. There are abundant abrupt occurrences of small cliffs locally, but the only extensive cliffs are on the northwest face adjacent to the Richelieu River. Here a solid rock wall rises precipitously with an extensive boulder talus at its base. Smaller cliffs crop up sporadically and are usually accompanied by basal talus.

Geology

The geological history of the Monteregians has not been satisfactorily explained. Volcanic origin was previously accepted but more recent examination of rock composition has indicated a lack of similarity to the fine-grained structure of quickly cooled volcanic materials. The igneus nature of the rocks is nevertheless clearly apparent. Different techniques of aging have been applied, some of which place them as Carboniferous (350 million years) and others as Tertiary (60 million years) (Clark 1952). The same author (personal communication) favors the later period of origin. The lack of fossil materials and the destructive processes of glacial wasting and erosion and subsequent extensive weathering are serious deterrents to attempts to age and clearly understand the rocks of the Monteregian series.

Using specific mineralogical types as a basis, the mountain may be roughly divided into two sections. The north and south cols separate a northwestern area composed predominantly of essexites and a southeastern area of nepheline sodalite syenite bedrock (O'Neill 1914). Locally there occur outcroppings of these rocks or variants of them such as yamaskite or rouvillite (type rocks of the Monteregian series), as well as surface deposits of limestone. Intrusions of essexite are found in the nepheline sodalite syenite at the line of junction of the two main types, to form a breccia. This would indicate a different period of origin for the main rock bodies and points to the intrusion of the syenite after the essexite. The intrusions of the hot igneus plugs through the lowland plain baked the paleozoic limestones and produced a hornfels collar that is found ringing the terrace areas (Clark 1952; O'Neill 1914).

The shallow depth of the soil mantle on many of the summits is evidence of the eroding effects of Pleistocene glaciation. The Monteregians standing isolated on a flat plain offered strong resistance to ice movements and were probably severely disturbed because of their position. The unequal splashing and deposition of drift in the periphery of the basin and other depressions and the preponderance of drift deposits adjacent to the south face, as well as the erratics scattered from place to place, offer additional evidence of glacial disruptions.

The recession periods also effected changes in the structure of the mountain. As the ice masses receded northward in the lowlands they continued to block

the St. Lawrence Valley drainage channel and the glacial melt waters collected in the upriver areas forming an extensive glacial lake that has since receded to what is in the present period Lake Champlain. The summits of the Monteregians projected as islands from this lake and during submergence stages gravel beaches and sand deposits were laid down. These beaches, although indistinct, can be detected at the present 575-ft level. Some of these gravel terraces are presently being exploited.

These geological processes have had considerable influence in producing the type of environments that presently exist for plants on the mountain, and these effects in association with the peculiar topographic features of the Monteregians must have also influenced the distributional patterns of many plants in the St. Lawrence Lowlands.

Soils

The critical effects of glaciation are most evident in having produced a very unequal distribution of the soil mantle. The deepest drift deposits occur around the inner rim of the basin and as talus at the base of the outer slopes. Generally as the tops of the different peaks are approached, the soils become gradually shallower. All of the tallest summits have a shallow soil layer and often frequent rock outcrops. The depth of the soil mantle as well as the origin of the materials has a significant effect on the types of forest communities which can become established as well as on the form and magnitude of tree growth.

Loam or sandy loam soils predominate over much of the area with the exceptions of sandy deposits which occur on the outer talus border and alluvial muck deposits which are confined to the depressions, cols, and low-lying areas adjacent to the lake. All of the soil profiles examined displayed characteristic traits indicating the process of podsolization. The development of an A_2 ashy-gray layer varied from one locality to another depending on the forest cover and other environmental factors. In some situations this was a clearly marked layer and in others it was present only as scattered gray silica particles.

The peculiar differentiation of the underlying bedrock into two distinct regions on the mountain would perhaps be expected to be reflected in the mineralogical characteristics of the soils overlying these different strata. During the scientific investigations a soil survey conducted by the Department of Geology of McGill University (Webber 1960) obtained interesting results in this regard. The overlying soils were found to reflect the composition of the underlying rock and those soils over the nepheline sodalite syenite area were richer in the elements rubidium, niobium, and zirconium, whereas those soils of the essexite contained significant, higher levels of strontium. Although considerably more work is required in this field and although the elements mentioned are seldom critical for plant growth, the results do indicate that significant mineralogical differences do exist in the soils of the two areas of different bedrock.

Climate

A series of climatic data for Mont St. Hilaire are not available. Installations for the recording of weather data have not been positioned on the summits

of any members of the Monteregian group and therefore there is no information that can be used to determine whether climatic conditions on the mountains deviate from those of the surrounding lowlands as they undoubtedly do.

During 1960 weather data were recorded at Lac Hertel on the south shore. As these data accumulate, a comparison of the climate at this altitude (570 ft) with the lowland region will be available. It would also be useful to take records on the summits of one of the higher peaks to aid in these comparisons.

The present lack of weather data for these locations precludes any consideration of the correlation between vegetation and climatic factors, and even if such were available it would be impossible to draw comparisons without many additional installations on the various exposures and in different topographic situations. This in itself is a major undertaking, but one that would be particularly rewarding on the mountain where a great variety of different situations are available in a relatively small area. Gross differences occur in the forest types on different exposures and the composition of forests at different altitudes, and these would serve as a gauge for climatic correlations if a study of this kind were undertaken. Considerable work has been completed in an attempt to ascertain vegetational differences in relation to exposure and altitude but these results will be reported in a subsequent paper.

In attempting to correlate climatic influences and vegetational differences on the mountain, the effects of the prevailing winds are an important consideration, as well as the effect of exposure on insolation. The preponderance of hardwood-conifer forests which become established in drier moisture situations, on south- and west-facing slopes, and the greater occurrence of hardwood forests which are most frequently found in moist situations, on north- and east-facing slopes, emphasize the importance of the prevailing west and southwesterly winds in creating drier conditions on the west- and south-facing slopes.

To present a generalized picture of climatic conditions in the region, the climatic data available for St. Hubert Airport, which is situated 15 miles to the southwest, are provided in Table II. Most of the averages are based on a

TABLE II

Climatic data for St. Hubert Airport

Average annual rainfall			28.15 in.
Average annual snowfall			92.0 in.
Average annual precipitation			37.35 in.
Mean annual temperature			42° F
Mean annual maximum temperature			51° F
Mean annual minimum temperature			33° F
Frost-free periods (average)			137 days
Frost-free period (longest)			160 days
Frost-free period (shortest)			123 days
Prevailing winds (average percentage frequency)			
S	20	Calm	3
SW	20	NE	7
W	13	E	5
NW	10	SE	6
N	16		

10-year period. The wind records are particularly valuable since there are good installations for measuring wind speed and direction and the station is in an exposed position in the lowlands at an elevation of 102 ft.

Lac Hertel

The lake originates in the three streams of the cols referred to previously and the drainage from an extensive seepage area along the west shore. During the spring runoff many sporadic streamlets originate in low-lying areas but these cease to exist later in the spring and are usually not evident as early as the first part of June.

The lake is a veritable paradise for the limnologist and the aquatic biologist. All of the lake waters find their source in the watershed of the inner bowl of the mountain. The lack of an inflowing stream precludes the possibility of pollution and the lake remains as a natural self-contained unit, ideally suited to the study of ecosystems (Maycock 1959*a*).

An area of approximately 75 acres is occupied by the lake. The waters are not deep; the deepest soundings recorded have been 23 ft. The bed consists of black organic muck with some fine to medium sand (Hare *et al.* 1959).

The only outflow at the south end is regulated by a dam that permits some control over the water level. The maximum level (572-ft elevation) is attained in April, and during the summer and fall of 1959 this receded to the 565-ft level.

The shallow nature of Lac Hertel and the absence of any rapid influx or outflow of water contributes markedly to the accumulation of organic materials and alluvial muck on the lake bed. The waters are rich in minerals and are alkaline as indicated by measured pH values ranging from 7.6 to 7.9. This rich supply of nutrients, the suitable substrate conditions, and a deep penetration of light permitted by clear water and a shallow bottom, all contribute to the production of a luxuriant aquatic vegetation. The predominant vegetation is submerged and bottom-rooted, but in areas of suitable depth, beds of floating aquatics have become established. The same growing conditions are favorable also to the build-up of algal populations.

Vegetation

The relative inaccessibility of many areas on Mont St. Hilaire and a long period of private ownership provides an explanation for the present untouched state of much of the vegetation. Hardwood stands are to be found on the slopes and summits of the more inaccessible peaks of the eastern half where no human disturbance of any kind is evident. Stands of the lower slopes in this location are luxuriant but great age and size are not characteristic of the stands at higher elevations since the tree species involved are growing under severe environmental conditions imposed by their situation. This results in a comparatively rapid turnover, and old individuals are very susceptible to diseases and the often catastrophic effects of wind. These forests are nevertheless a spectacular sight to behold and a novelty in the long-settled St. Lawrence Valley. Many of the low-lying areas where growth does attain considerable proportions, especially those of the main valley, have been subjected to selective logging.

Regional Vegetational Patterns

To place the forest vegetation of the Monteregians and particularly of St. Hilaire Mountain in proper perspective, and to understand the composition of the forests in different environmental situations, it is first necessary to consider the forest conditions of the region of extreme southern Quebec. In the St. Lawrence Valley and the adjacent Ottawa River Valley the prevailing type of mature forest encountered on upland situations of moderate moisture is composed of deciduous species of *Acer saccharum*, *Fagus grandifolia*, and *Tilia americana*. Occasionally the conifers *Pinus strobus* and *Tsuga canadensis* do occur as scattered individuals with the deciduous elements but this is increasingly characteristic of situations of excessive soil drainage. Dansereau (1959) claims this forest community is the climatic climax for the St. Lawrence Lowlands, particularly areas below 1000 ft, but emphasizes the absence of conifers.

In environmental situations of increased moisture, forests of *Ulmus americana*, *Acer rubrum*, *Betula lutea*, *Acer saccharinum*, *Fraxinus americana*, and *F. nigra* frequently occur while the drier uplands support stands of *Quercus rubra*, *Pinus strobus*, and *Ostrya virginiana*. *Acer saccharum* and *Fagus grandifolia* occur with these species mentioned, in various combinations and admixtures, and remain important except where extremes of moisture prevail. Many other tree species, some of which are on the fringe of their northern limit and which are found more frequently in the St. Lawrence Lowland region as it passes into southern Ontario, also grow in the forests of the area but seldom as significant dominants. These include *Fraxinus pennsylvanica*, *Carya ovata*, *C. cordiformis*, *Juglans cinerea*, *Prunus serotina*, *Quercus alba*, *Q. bicolor*, *Q. macrocarpa*, *Ulmus rubra*, and *Ulmus thomasi*.

The sporadic bogs of the St. Lawrence Lowlands are covered with forests of *Picea mariana*, *Larix laricina*, *Abies balsamea*, *Betula papyrifera*, *B. populifolia*, and *Thuja occidentalis*. Areas which have been subjected to fire or extensive logging or other disturbances support secondary forests dominated by *Betula papyrifera*, *B. populifolia*, *Populus tremuloides*, and *P. grandidentata* in pure stands or various mixtures, as well as second growth stands of the hardwood species already referred to.

The transitional nature of the forest communities of this lowland region should be emphasized. More-northern species are of sporadic occurrence over much of the region and become increasingly important in more-northern sections of the Lowlands and as the Laurentians and Appalachians are approached.

Vegetation of Mont St. Hilaire

Mont St. Hilaire is centrally situated in the midst of this transition area. It is because of this situation that forest ecological studies here present a challenge but are rewarding from the point of view of the value of the results obtained. If this position creates climatic and other environmental modifications that are more drastic than the environs, then these changes would be expected to be reflected in forest composition similar to that of more northerly situated regions. Conversely, if an amelioration of the prevailing conditions occurs, then more-southern forest communities would be anticipated. The

variable environmental features of the mountain might be expected to produce vegetational variation of great complexity and this in turn would present a challenge to studies directed toward the interrelations of forest vegetation and environmental factors. Thus the situation in a transitional area coupled with the variable environmental situations presents a remarkably complex ecological situation in a relatively limited area of perhaps 6 square miles (Maycock 1959*b*).

The transitional aspects are reflected in forest composition. In terms of broad relationships and compositional similarity, the forests are best described as northern conifer-hardwoods. This general description overshadows much of the variation that actually exists in the composition of the vegetation of different localities, however. The forests of the lower levels, particularly below 1000 ft, tend to be predominantly deciduous in character, while those of higher altitudes contain varying amounts of conifers. Actual composition in terms of broad-leaved and coniferous trees is dependent on the site characteristics of the locality and not necessarily on its altitude. Thus stands of pure hardwood forest occur on some of the highest peaks and stands of hardwood-conifers at lower elevations.

Exposure is one of the site characteristics that exerts strong influence in determining forest composition. This impression is gained by observing the tree cover on the slopes of the peaks that are in view from the south shore of Lac Hertel looking northward. Even casually it is evident that there are decided tendencies for forests composed predominantly of deciduous elements to grow on north- and east-facing slopes and for mixed coniferous–deciduous types or nearly pure coniferous types to become established on south- and west-facing slopes.

Stands of *Acer–Fagus* or *Fagus–Acer* predominate on the north- or east-facing slopes. The south- and west-facing slopes tend to support stands of *Acer–Quercus*, *Quercus–Acer*, *Quercus–Pinus*, or *Pinus*, all with admixtures of other tree species. The summit areas are forested largely in relation to the depth of the soil mantle available for tree growth. *Acer–Fagus* forest may occur to an altitude of 1350 ft where the soils are deep and sufficient moisture is available. Scrubby stands of *Quercus–Acer* or *Quercus–Pinus* are found on those summits where soils are shallow, often with frequent rock outcrop, and moisture conditions are dry.

Moist depressions are infrequent on the mountain and occur in cols and ravines between adjacent peaks. These habitats predominate in the low areas which surround the lake, in the depressions of the main creeks which feed the lake, and in certain places where seepage systems lie near the ground surface. Stands composed of *Ulmus americana*, *Fraxinus nigra*, *Betula lutea*, *Thuja occidentalis*, *Acer rubrum*, *Tsuga canadensis*, and *Juglans cinerea* in various combinations are typical in these situations. In these restricted habitats a scattering of *Abies balsamea* may be present as well as *Acer saccharum*.

These descriptions have briefly dealt with the composition of forests growing on sites where conditions of moisture, soil, and topography are extreme. Many areas exist that represent intermediate conditions with respect to these and many other habitat characteristics, and in such areas

almost every conceivable variety of forest composition of the types described or types transitional between them is possible. Of the major forest types of the St. Lawrence Valley, only bog forests do not occur on the mountain.

Forests comprise the greatest proportion of the total plant cover. This is natural in a region where climatic conditions favor the ultimate establishment of forest. However, in certain locations the present environmental conditions support a lesser community. Such communities occur in the areas surrounding the lake and adjacent to the creeks, particularly as they enter the lake. These include the beach communities, wet shrub communities and marshes, and the emergent, floating, and submerged aquatic communities. In disturbed areas secondary forests, shrub communities, open weed communities, and cliff communities are prevalent.

Many of these minor plant communities are related to some type of disturbance. Some of the areas have been selectively logged in the past, particularly the hardwood stands at low levels surrounding the lake. Patch fires, usually of a restricted nature, have produced open areas for colonization which subsequently give way to secondary forests. Other disturbance communities are found near roads, trails, a small orchard, and around a few dwellings that have been constructed on the south shore of the lake. All of the minor communities and those related to disturbance are important from the point of view of the different species of plants that are responsible for their vegetational structure. Although these communities are relatively limited in area when compared with the forests, they still possess different plants that seem as important as the typically widespread and abundant forest species, in the floral list.

Floristic Survey

Previous Studies of the Flora

It is disappointing to find that little research of a botanical nature has been accomplished in an area that is so rich in natural history phenomena and which has excited the interests of scientists and amateurs for so long a period. As early as the 1870's Sir William Dawson, principal of McGill University between 1855 and 1893, led field trips of the Montreal Natural History Society to St. Hilaire Mountain to study geology and botany and published remarks on his studies in the Canadian Naturalist (Dawson 1878).

The first extensive collections to be taken on a member of the Monteregian group were those of Andrew F. Holmes, First Professor of Botany in McGill University. He collected plants on Mount Royal as early as 1820 and prepared an extensive herbarium of almost 600 sheets (Barnston 1859). Later, in 1884, Henry H. Lyman, a Montreal druggist and a member of the Montreal Natural History Society, made extensive collections on Mount Royal and on Mont St. Hilaire. In the years following this period until about 1915 the Montreal Natural History Society actively collected in southern Quebec but neglected to collect specifically on the Monteregian Hills.

The French scientists and naturalists in the intervening period have continued studies of the flora, particularly of the southern areas of the province, and this work resulted in the *Flore Laurentienne* by Brother Marie-Victorin in 1947 (Marie-Victorin 1947). Victorin collected on St. Hilaire in 1905 and in subsequent years (1912–1920). It is of special interest to note that many

of Brother Victorin's first 1000 sheets of plant specimens were taken on Mont St. Hilaire and that probably much of his early enthusiasm for the subject was imbued during "les herborisations sur la montagne de St. Hilaire" (M. Raymond, personal communication). He published a popular note on these excursions (Marie-Victorin 1913) in which he generally describes the vegetation and rich flora, particularly the vernals of the hardwood forests. Raymond (1950) in depicting the phytogeographical regions of Quebec briefly mentions the vegetational characteristics of St. Hilaire and emphasizes the distributions of a few oddities. He compares the flora of the mountain generally with other members of the Monteregian group, particularly Mount Johnson, where he had intensively botanized.

Apart from these studies, which have only briefly considered the vegetation and flora, there are additional collections that have been taken but which have not been published. Frère Cléonique of l'Institut des Frères de l'Instruction Chrétienne, a secondary school teacher in Montreal, collected extensively on St. Hilaire in 1938. Lionel Cinq-Mars (1959) has prepared an extensive list of the plants of Mont Rouge (Cinq-Mars 1959) and Claire Morin-Gauthier has taken specimens on Mont St. Bruno.

The sparse literature on the flora, vegetation, and other natural phenomena of the Monteregian Hills is disappointing considering the rich materials that are available. There is an acute need for this work to proceed while there are at least partially undisturbed areas available.

The Known Existing Flora

During the summer of 1959 and subsequently throughout most of the growing season of 1960, extensive collections were taken in all vegetation types on the mountain and in conjunction with ecological studies concerned with vegetational composition. The majority of these efforts were concentrated on the mountain proper, particularly in areas above the lower terraces or above the 400-ft altitudinal level. The lower terraces often stretch a considerable distance from the mountain and include extensive areas that lie outside the McGill holdings. Since most of these terraces are under apple culture or are otherwise cleared or disturbed they were avoided in the studies. The flora and vegetation of the mountain proper are the areas that will be used for future studies and this separation was therefore reasonable. The inclusion of these terrace areas would only have added greatly to the list of introduced species.

An attempt was made to collect a voucher specimen of all plant species observed, but in some cases this was not accomplished. A few of the more common species were missed, but all taxonomically difficult species were taken. In the two summers a total of 500 different higher plants including the fern and fern-allies were observed, and of these all but 43 were collected and pressed. In addition to these some 25 additional species which were collected on Mont St. Hilaire by other workers have been observed either in the McGill College Herbarium, the Herbarium of the Institute of Botany, University of Montreal, or the Herbarium of the Montreal Botanic Garden. In all, a total of 526 different species, 22 varieties, and 3 forms or a total of 551 different entities are present on the mountain. These have been broken down into

TABLE III

A synopsis of the taxonomic groupings comprising the vascular flora of Mont St. Hilaire

	Species	Varieties	Forms	Total
Lycopsida	9			9
Sphenopsida	5			5
Pteropsida	30	4		34
Spermatophyta				
Gymnospermae	8	1		9
Angiospermae				
Monocotyledoneae	125	8	1	134
Dicotyledoneae	349	9	2	360
Total	526	22	3	551

various taxonomic categories, as presented in Table III.

The 551 entities represent a variety of life-form habits. Of the group, 34 are trees, 65 are shrubs, 6 are lianas, and 421 are herbs of various kinds. Twenty-three species are more or less restricted to aquatic environments or are only found on land because of fluctuations in water levels. Thus a variety of ecological characteristics are represented in the flora.

With regard to taxonomic statistics the plants are included in 88 different families with 9 of these including fern-allies, ferns, and conifers, 14 including monocotyledons, and 65 of dicotyledonous origins. The family represented by the largest number of species is the Compositae (11%) and this is followed in order by Rosaceae (7%), Cyperaceae (7%), Gramineae (7%), and Ranunculaceae (3%). The ferns, including members of 3 families, comprise a total of 30 species (6%). The number of introduced species on the mountain is not excessively high (57) and it comprises only 11 per cent of the total flora.

In addition to the higher vascular plants, mosses, lichens, algae, liverworts, and fungi occur profusely in the many different plant communities. A wealth of fungi are found on the mountain, and throughout the growing season a progression of the fruiting bodies is produced following moist periods. In shaded environments the moss communities may comprise a prominent part of the vegetation. The number of these different organisms cannot presently be estimated. It was impossible to attempt to collect and identify these plants in the time available and specialists in these fields should be attracted to St. Hilaire to assist in this task. A start has been made in accumulating information on the moss flora with the assistance of Mr. J. Kucyniak of the Montreal Botanic Garden.

Concerning the possible number of higher plants in the total flora of the mountain it is difficult to speculate. There is little doubt that new species will be discovered now that a flora is available. No serious effort was made to botanize exhaustively in disturbed areas and many of the plants which will be added in future will be introduced species that are often sporadic in their occurrence. There are also a few areas, particularly the steep cliffs of the north face, that have not been extensively covered, but in general the native flora of the mountain should be well represented in the present flora. Some

insight into this problem can be obtained by comparing the flora of Mont St. Hilaire with the floras of other members of the Monteregian Group that are presently known. Holmes collected some 550 different species on Mount Royal in the early 1820's, and although few introduced species would have been present there at that time, this probably was not a complete flora. More recently Cinq-Mars (Cinq-Mars 1959) has an accumulated list of almost 750 species for Rougemont, which is a larger mountain and more extensively disturbed. His list includes also plants of the lower terraces and environs and a larger percentage (18%; approximately 133 species) are introduced. Raymond (personal communication) has botanized extensively on Mount Johnson, the smallest member of the group, and estimates a total flora of 500 species. If these statistics are meaningful in relation to the sizes and extent of disturbance on the various members, a flora of perhaps 650 higher vascular species may be expected ultimately for Mont St. Hilaire.

Affinities of the Flora

With the exception of the introduced species, the plants comprising the flora of Mont St. Hilaire are those which commonly occur throughout the Northern Conifer-Hardwood region and the Northern Hardwoods region of eastern North America. A few species which attain greatest ecological importance in the Boreal Forest are also present but frequently these are confined to more-restricted habitats. The central position of St. Hilaire and the Monteregians generally, in a region where these major plant formations tend to become confluent, has resulted in a diverse and comparatively rich flora. The abundant and variable environmental situations available are in large measure responsible for the rich flora because of the diversity of ecological opportunities that these habitats present for plant establishment and growth.

In describing the phytogeographical regions of Quebec, Raymond (1950) includes Mont St. Hilaire within the Monteregian Province, which is a floristic subunit of the Laurentian Region. This expresses again the Northern Conifer-Hardwood affinity referred to previously. This floristic province is considered to bear a relationship to the Richelieu Valley Province within which many of the Monteregians occur. This in turn reflects the Northern Hardwood tendencies in the flora. These phytogeographical regions are based on the distributions of particular groups of plants which appear to be more or less restricted to particular physiographic regions, although a large majority of the species comprising the flora of southern Quebec may range throughout several of the provinces.

In the total flora there are groups of plants which have apparent distributional characteristics in common which support these tendencies. *Hamamelis virginiana*, *Staphylea trifolia*, *Dryopteris hexagonoptera*, *Athyrium pycnocarpon*, *Prunus serotina*, and *Vaccinium corymbosum* represent species which are common in southern Ontario, and here are on the northern edge of their range. Species such as *Linnaea borealis*, *Clintonia borealis*, *Cornus canadensis*, *Taxus canadensis*, *Potentilla tridentata*, *Draba arabisans*, and *Pyrola asarifolia* occur most frequently in boreal forest communities and in southern Quebec are approaching their southern lowland limits. Marie-Victorin (1947) recognized the northern conifer-hardwood and boreal affinities

of members of the flora of St. Hilaire and drew particular attention to them:

> "Les roches éruptives qui constituent les Montérégiennes, bien que fortement basique, portent une flore analogue à celle des Laurentides et des Adirondacks. Leur couverture végétale est la forêt mixte, et les types calcicoles manquent presque totalement. Quand les sommets sont dénudés ce qui arrive rarement on trouve quelques représentants d'une flore plus boréale."

Other plants that are most typical of the deciduous forests are widespread on St. Hilaire. *Erythronium americanum*, *Trillium grandiflorum*, *Dicentra cucullaria*, *D. canadense*, *Thalictrum dioicum*, *Allium tricoccum*, and *Caulophyllum thalictroides* are a few representatives of the vernal herbs in these forests. The majority of the native plants of the mountain may be conveniently included in one of these three major categories of affinity although a large number might perhaps be better recognized as broad-ranging species, having important occurrence in more than one of these vegetational formations.

It is impossible to discuss the origins of the floral elements of the mountain without considerably more knowledge of the paths of migration which carried the constituents of the flora of southern Quebec to their present positions in postglacial periods. There should be little doubt, however, that the members of the Monteregian group must have been of considerable importance in the early influx of plants into this region and in the shuffling that must have occurred between glacial substages. Under presently existing climatic conditions there are marked differences in local microclimate on the various faces and between the summits and protected depressions, and these differences are reflected in markedly different forest composition. This has already been discussed under the section on climate. If this is true to-day, then it is interesting to speculate upon the manner in which ameliorated or more drastic conditions in localities on the mountain may have affected the entrance and survival of plants in postglacial periods and then subsequently provided a source for the reclamation of surrounding areas. This discussion is not intended to imply that the Monteregians served as refugia during the height of glacial conditions, but is offered as a suggestion of the possible way in which these areas may have assisted in the recovery of vegetation in the lowland region subsequent to the retreat of ice.

The peculiar patterns of plant distribution on members of the Monteregian Group, particularly the common occurrences of particular species on some or all of the members and especially species with peculiar distributions in the area of southern Quebec (as, for example, *Potentilla tridentata*, *Selaginella rupestris*, *Draba arabisans*, *Camptosorus rhizophyllus*, *Hamamelis virginiana*, *Dryopteris hexagonoptera*, *Sanicula trifoliata*, *Desmodium nudiflorum*, *Conopholis americana*, *Allium schoenoprasum*, *Panax quinquefolius*, *Podophyllum peltatum*, and *Staphylea trifolia*, and many others), offer considerable support for this assumption. The solution of these problems is dependent upon more information concerning the exact distributions of the elements in the flora of southern Quebec.

The Floral List

In the accompanying list (i.e., the Appendix), the higher vascular plants

presently known to occur on Mont St. Hilaire, particularly above the 400-ft level of altitude, are presented, grouped according to families. The nomenclature and order of families followed is that of Gray's Manual of Botany, 8th edition (Fernald 1950).

Short notes are provided concerning general habitat, moisture conditions of the site, exposure, and other relevant information, particularly the community or vegetation type of optimum occurrence. These ecological preferences are based on quantitative data obtained in sampling stands of the forest types and are therefore quantitatively accurate and not simply the results of general observations. Some information about the relative abundance of the species is presented and this refers more specifically to the abundance of the plant in situations where it attains its ecological optimum and not its general abundance on the mountain, although for species of broad ecological tolerance these characteristics are not exclusive. Species for which specimens are available in the McGill College Herbarium of McGill University are preceded by an asterisk, and those species which have been collected by other workers are followed by the citation of their specimens.

These collections taken on Mont St. Hilaire will be of value in future scientific research on the mountain and in furthering our knowledge of the flora of southern Quebec but they also signify a historical event. Andrew F. Holmes was one of the first botanists to collect on the Island of Montreal and particularly Mount Royal—a Monteregian representative. He took his first plant specimens there in 1820, and later in 1856 presented his entire herbarium of more than 600 species to McGill College. This collection formed the nucleus of the present McGill College Herbarium which has been enlarged to include more than 50,000 sheets. This herbarium is presently the recipient of extensive collections taken on another member of the Monteregian Group—Mont St. Hilaire—but after the lapse of more than 140 years.

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Appendix

Annotated List of Species

EQUISETACEAE

**Equisetum arvense* L.: Common in rich wet-mesic and wet woods and in marshes.

Equisetum fluviatile L.: Marshes adjacent to Lac Hertel and wet woods. Infrequent.

**Equisetum hyemale* L.: Occurring on the fringe of wet woods adjacent to the lakeshore and in dry-mesic woods. Growing in extensive clones. Common.

**Equisetum pratense* Ehrh.: Wet-mesic and wet woods. Common.

**Equisetum sylvaticum* L.: Common in wet and wet-mesic woods.

LYCOPODIACEAE

**Lycopodium annotinum* L.: Dry pine and hemlock woods. Infrequent.

**Lycopodium clavatum* L.: Wet-mesic pine and hemlock woods. Common.

**Lycopodium complanatum* L.: Dry coniferous stands particularly in openings. Rare.

**Lycopodium lucidulum* Michx.: Mesic, wet-mesic, and wet woods: particularly abundant in stands of this type adjacent to streams. Very common.

**Lycopodium obscurum* L.: Dry-mesic, mesic, and wet-mesic woods. Common.

Lycopodium tristachyum Pursh: Dry pine–oak woods. Rare.

SELAGINELLACEAE

Selaginella rupestris (L.) Spring: Dry woods and rocky open situations, often on boulder tops. Rare.

*Specimens available in McGill College Herbarium of McGill University.

ISOETACEAE

Isoetes macrospora Dur.: Marsh flats and riparian habitats of Lac Hertel (Victorin No. 769, 1914). Not common.

**Isoetes riparia* Engelm.: In water or riparian habitats along lakeshore. Not common.

OPHIOGLOSSACEAE

**Botrychium dissectum* Spreng.: Mesic to wet woods. Rare.

**Botrychium lanceolatum* (Gmel.) Angstr.: Wet, ground adjacent to creeks. Rare.

**Botrychium multifidum* (Gmel.) Rupr.: Wet, low ground in woods. Rare.

**Botrychium virginianum* (L.) Sw.: Most abundant in mesic and wet-mesic hardwoods. Common.

**Botrychium matricariaefolium* A. Br.: Mesic to wet woods. Rare.

OSMUNDACEAE

**Osmunda cinnamomea* L.: Lowland swamp forest and woodland swamp depressions. Common.

**Osmunda claytoniana* L.: Lowland woods and wet openings. Common.

**Osmunda regalis* L.: Common in marshes and swamps.

POLYPODIACEAE

**Adiantum pedatum* L.: Most frequent in mesic and wet-mesic hardwoods, but occurring also in dry-mesic and wet woods. Common.

**Athyrium filix-femina* (L.) Roth: Wet-mesic and wet woods. Common.

**Athyrium pycnocarpon* (Spreng.) Tidestr.: Wet-mesic hardwoods. Very rare, in only two localities.

**Athyrium thelypterioides* (Michx.) Desv.: Mesic and wet-mesic woods. Infrequent.

**Cystopteris bulbifera* (L.) Bernh.: Infrequent in mesic to wet woods. Adjacent to creeks, on wet slopes and seeping cliffs, particularly in areas of limestone outcrop.

**Cystopteris fragilis* (L.) Bernh.: Common in hardwoods in all moisture situations.

Dennstaedtia punctilobula (Michx.) Moore: Uncommon in lowland swamps. (Cleonique No. 10472, 1938.)

**Dryopteris cristata* (L.) Gray *var. clintoniana* (D. C. Eat.) Underw.: Rich wet woods. Very rare.

**Dryopteris disjuncta* (Ledeb.) C. V. Mort.: Common in wet-mesic and wet woods.

**Dryopteris goldiana* (Hook.) Gray: Rare in mesic, wet-mesic, and wet woods.

**Dryopteris hexagonoptera* (Michx.) Christens.: Wet-mesic and wet hardwoods, especially near creeks. Rare.

**Dryopteris marginalis* (L.) Gray: Very common in dry, dry-mesic, and mesic woods. Frequently associated with outcrop or shallow soils.

**Dryopteris noveboracensis* (L.) Gray: Quite common in wet and wet-mesic woods.

**Dryopteris phegopteris* (L.) Christens.: Wet-mesic and wet woods. Common.

**Dryopteris spinulosa* (O. F. Muell.) Watt: Abundant in all forest types of the mountain. The commonest fern of the area.

**Dryopteris spinulosa* (O. F. Muell.) Watt *var. intermedia* (Muhl.) Underw.: Mesic and wet-mesic woods. Common but more restricted ecologically than the typical form.

**Dryopteris thelypteris* (L.) Gray *var. pubescens* (Lawson) Nakai: Wet and wet-mesic woods, particularly lowland swamp hardwoods. Infrequent.

**Onoclea sensibilis* L.: Very common, particularly in wet woods.

**Polypodium virginianum* L.: Common in dry and dry-mesic woods, especially coniferous woods. Locally abundant on cliffs and dry boulder tops.

**Polystichum acrostichoides* (Michx.) Schott: Rich mesic and wet-mesic hardwoods. Common.

**Pteridium aquilinum* (L.) Kuhn *var. latiusculum* (Desv.) Underw.: Infrequent in dry coniferous and mixed conifer-hardwoods.

**Pteretis pensylvanica* (Willd.) Fern.: Wet and wet-mesic woods and lowland swamp depressions. Infrequent.

**Woodsia ilvensis* (L.) R. Br.: Restricted to dry woods on exposed slopes and on outcrops and cliff crevices. Infrequent.

TAXACEAE

**Taxus canadensis* Marsh: Abundant particularly in wet-mesic conifer-hardwoods but occurring also in wet woods.

PINACEAE

**Abies balsamea* (L.) Mill.: Attaining greatest ecological importance in wet lowland conifer-hardwoods adjacent to creeks. Infrequent except in these situations.

**Juniperus communis* L. *var. depressa* Pursh: Dry conifer-hardwoods on dry rocky west-facing slopes. Rare.

**Picea glauca* (Moench.) Voss: Dry woods. Very rare; only two mature trees observed on the summit of the Sugar Loaf.

**Pinus resinosa* Ait.: Locally abundant in dry conifer and conifer-hardwood stands, particularly on south- or west-facing slopes.

**Pinus strobus* L.: Common particularly in dry stands with a southern or western exposure. Frequent on dry knolls fringing Lac Hertel.

**Thuja occidentalis* L.: Common in wet lowland or swamp forests and occurring infrequently as a shrub or sparse tree on dry, rocky slopes.

**Tsuga canadensis* (L.) Carr.: Abundant in dry, wet-mesic, and wet conifer-hardwoods. A conflict of ecological requirements is presented by the species in that it occurs on dry rocky summits, in wet swamps or lowland situations, and on slopes, particularly on those with eastern exposures.

TYPHACEAE

**Typha latifolia* L.: Infrequent and restricted to marshes at the north end of Lac Hertel.

SPARGANIACEAE

**Sparganium chlorocarpum* Rydb.: Marsh border of the lake. Infrequent.

**Sparganium eurycarpum* Engelm.: Rare, in marshes.

**Sparganium androcladum* (Engelm.) Morong: Infrequent, in marshes.

ZOSTERACEAE

**Potamogeton gramineus* L.: Submerged aquatic in Lac Hertel. Infrequent.

**Potamogeton robbinsii* Oakes: Submerged aquatic. Common.

**Potamogeton richardsonii* Rydb.: Submerged aquatic. Very abundant.

**Potamogeton spirillus* Tuckerm.: Submerged aquatic. Very abundant.

NAJADACEAE

**Najas flexilis* (Willd.) Rostk. & Schmidt: Submerged aquatic. Very abundant in the lake.

ALISMATACEAE

**Alisma triviale* Pursh: Marshes. Common.

**Sagittaria latifolia* Willd.: Marshes and adjacent swamp forests. Common. (Lyman, 1877.)

HYDROCHARITACEAE

**Vallisneria americana* Michx.: Submerged or floating aquatic. Abundant in the lake.

GRAMINEAE

Agropyron repens (L.) Beauv.: Common in disturbed habitats. Orchards.

**Agropyron trachycaulum* (Link) Malte: Dry forests. Uncommon.

**Agrostis perennans* (Walt.) Tuckerm.: Common in dry hardwoods.

**Agrostis scabra* Willd.: Dry, open grassy summits. Common.

**Brachyelytrum erectum* (Schreb.) Beauv.: Mesic, wet-mesic, and wet woods. Along paths and in openings. Common.

**Brachyelytrum erectum* (Schreb.) Beauv. *var. septentrionale* Babel: Hardwoods. Infrequent.

**Calamagrostis canadensis* (Michx.) Nutt.: Marsh communities, especially shrub stages, and lowland swamp hardwoods. Infrequent.

**Cinna latifolia* (Trev.) Griseb.: Mesic and wet-mesic hardwoods. Infrequent.

**Danthonia spicata* (L.) Beauv.: Dry hardwoods and conifer-hardwoods. Common.

**Deschampsia flexuosa* (L.) Trin.: Dry woods. Infrequent.
Digitaria ischaemum (Schreb.) Muhl.: Disturbed areas. Common. Introduced species.
**Echinochloa crusgalli* (L.) Beauv.: Disturbed areas. Infrequent. Introduced.
**Echinochloa pungens* (Poir.) Rydb.: Disturbed areas. Infrequent.
**Glyceria canadensis* (Michx.) Trin.: Open, marshes. Rare.
**Glyceria melicaria* (Michx.) F. T. Hubbard: Lowland swamp forest. Rare.
**Glyceria striata* (Lam.) Hitchc.: Abundant in wet and wet-mesic forests.
**Hystrix patula* Moench.: Dry to mesic woods. Infrequent.
**Hystrix patula* Moench. *var. bigeloviana* (Fern.) Deam: Similar habitats. Common.
**Leersia oryzoides* (L.) Sw.: Marshes and newly formed riparian habitats. Abundant.
**Milium effusum* L.: Frequent in woods, particularly mesic hardwoods.
**Oryzopsis asperifolia* Michx.: Abundant in dry and dry-mesic woods.
**Oryzopsis racemosa* (Sm.) Ricker: Dry to mesic woods. Frequent.
**Panicum capillare* L.: Abundant in marshes and gravel beaches.
**Panicum tuckermani* Fern.: Marshes. Infrequent.
**Panicum latifolium* L.: Dry woods. Common.
**Phalaris arundinacea* L.: Lowland swamp hardwoods and marshes. Uncommon.
Phleum pratense L.: Disturbed situations. Common in the orchard. Introduced.
**Poa annua* L.: Disturbed areas. Introduced.
**Poa compressa* L.: Dry woods and open summit meadows. Common. Introduced.
**Poa glauca* Vahl: Dry woods. Infrequent.
**Poa languida* Hitchc.: Mesic, dry-mesic, and wet-mesic woods. Common.
**Poa palustris* L.: Dry and wet hardwoods. Frequent.
Poa pratensis L.: Dry, open meadows and disturbed habitats. Common.
**Schizachne purpurascens* (Torr.) Swallen: Mesic hardwoods. Infrequent.
**Setaria glauca* (L.) Beauv.: Marshes. Common. Introduced.
**Setaria viridis* (L.) Beauv.: Marshes. Common. Introduced.
**Sphenopholis intermedia* Rydb.: Dry to mesic woods. Common.

CYPERACEAE

**Carex aenea* Fern.: Dry to mesic woods. Uncommon.
**Carex arctata* Boott: Common in woods, particularly dry-mesic hardwoods.
Carex backii Boott: Dry woods. (Victorin No. 717, 1914; Raymond No. 2829, 1953.) Uncommon.
**Carex communis* Bailey: Dry and dry-mesic conifer-hardwoods. Frequent.
**Carex comosa* Boott: Wet conifer-hardwoods. Rare.
**Carex convoluta* Mackenz.: Dry-mesic hardwoods. Infrequent.
**Carex crinita* Lam.: Wet, lowland swamp hardwoods. Common.
**Carex deweyana* Schwein.: Mesic and dry–mesic woods, but a widespread species in all forest types. Common.
**Carex gracillima* Schwein.: Infrequent in mesic hardwoods.
**Carex hitchcockiana* Dew.: Dry to mesic woods. Uncommon.
Carex houghtonii Torr.: Dry woods. (Victorin No. 727, 1914.)
**Carex hystricina* Muhl.: Wet hardwood-conifer woods. Rare.
**Carex lacustris* Willd.: Wet hardwood-conifer woods. Rare.
**Carex laxiflora* Lam.: Dry-mesic to wet-mesic woods. Common.
**Carex leptalea* Wahlenb.: Wet hardwood-conifer woods. Rare.
**Carex leptonervia* Fern.: Mesic and dry-mesic hardwoods. Infrequent.
**Carex normalis* Mackenz.: Wet lowland hardwoods. Infrequent.
**Carex pedunculata* Muhl.: A woodland sedge with a broad ecological tolerance. Attaining greatest importance in rich mesic hardwoods. Common.
**Carex pensylvanica* Lam.: Another species of broad tolerance. Abundant in dry and dry-mesic woods.

**Carex plantaginea* Lam.: Mesic and wet-mesic hardwoods. Infrequent.
**Carex platyphylla* Carey: Dry to mesic woods. Uncommon.
Carex prasina Wahlenb.: Marshes. Infrequent. (Cleonique No. 9962, 1938.)
**Carex radiata* (Wahlenb.) Dew.: Infrequent in mesic and wet-mesic hardwoods.
**Carex retrorsa* Schwein.: Wet, lowland swamp hardwoods. Not of common occurrence.
**Carex rosea* Schkuhr.: Mesic and wet-mesic hardwoods. Uncommon.
**Carex scabrata* Schwein.: Wet to wet-mesic conifer-hardwoods. Infrequent. (Cleonique No. 9538, 1937.)
**Carex sprengelii* Dew.: Dry to mesic hardwoods. Rare. (Raymond No. 2831, 1953.)
**Carex stipata* Muhl.: Wet swamp hardwood forest. Infrequent.
**Carex tenera* Dew.: Dry woods. Rare.
**Carex vesicaria* L.: Wet swamp forest. Rare.
**Cyperus inflexus* Muhl.: Newly exposed riparian habitats along lakeshore. Common.
**Cyperus rivularis* Kunth: Newly exposed mud flats along lakeshore. Common.
Dulichium arundinaceum (L.) Britt.: Marshes adjacent to lakeshore. Rare.
**Eleocharis acicularis* (L.) R. & S.: Marshes and wet mud flats. Common.
**Eleocharis obtusa* (Willd.) Schultes *var. jejuna* Fern.: Marshes and recently exposed riparian stands. Common.
**Eleocharis ovata* (Roth) R. & S.: Mud flats. Uncommon.
**Eleocharis smallii* Britt.: Emergent aquatic, west shore of lake. Rare.
**Scirpus atrovirens* Willd.: Damp grassy meadow in seepage area. Rare.
Scirpus validus Vahl: Emergent aquatic, south shore of Lac Hertel. Rare.

ARACEAE

**Arisaema atrorubens* (Ait.) Blume: Wet and wet-mesic woods. Common in all forest types except dry woods.

LEMNACEAE

Lemna minor L.: Floating aquatic of quiet shaded situations on the lake edge and marshes. Infrequent.

JUNCACEAE

**Juncus effusus* L. *var. solutus* Fern. & Wieg.: Marshes, west shore of lake. Rare.
Juncus subtilis Mey.: Marshes. Very rare. (Victorin, 1912.)
**Juncus tenuis* Willd.: Disturbed areas including newly exposed beaches and lake margins and open meadows on summits. Common.

LILIACEAE

**Allium schoenoprasum* L. *var. sibiricum* (L.) Hartm.: Steep, gravelly slopes of Sugar Loaf summit. Very rare.
**Allium tricoccum* Ait.: Common in rich, shaded, mesic hardwoods.
**Asparagus officinalis* L.: Openings in dry woods. Very rare, introduced weed.
**Clintonia borealis* (Ait.) Raf.: Wet to mesic woods. Attaining greatest ecological importance in wet conifer-hardwoods. Common.
**Erythronium americanum* Ker: Common in hardwoods, particularly mesic stands. Vernal species.
**Lilium tigrinum* L.: One small clump persisting on Sugar Loaf summit. Garden species, either introduced by birds or planted by pilgrims.
**Maianthemum canadense* Desf.: Possessing very broad tolerance, in all forested sites of the mountain. Very abundant and particularly so at the dry end of the forest continuum.
**Medeola virginiana* L.: Frequent in all types of woods, especially wet-mesic types.
**Polygonatum pubescens* (Willd.) Pursh: Broad ecological tolerance. Frequent in all forest types, particularly mesic maple–beech stands.
**Smilacina racemosa* (L.) Desf.: Abundant in all forested areas but attaining optimum importance in mesic and wet-mesic hardwoods.
**Streptopus amplexifolius* (L.) DC.: Common in maple–beech woods.

**Streptopus roseus* Michx.: Wet to dry-mesic woods, particularly abundant in mesic hardwoods and wet conifer-hardwoods.

**Trillium erectum* L.: Dry-mesic and mesic woods. Frequent.

**Trillium erectum* L. *forma viridiflorum* (Hook.) Peattie: Rich mesic beech–maple forest and wet lowland forest. Rare.

**Trillium grandiflorum* (Michx.) Salisb.: In all forest types but of greatest frequency in dry-mesic and mesic hardwoods. Very common.

**Trillium undulatum* Willd.: Dry coniferous woods and wet conifer-hardwoods. Rare.

**Uvularia grandiflora* Sm.: Wet-mesic, mesic, and dry-mesic hardwoods. Common.

**Uvularia sessilifolia* L.: Of widespread occurrence in forests of all types. Most frequent in dry-mesic and mesic woods.

IRIDACEAE

**Iris versicolor* L.: Wet lowland hardwoods, marshes, and wet woodland depressions. Infrequent.

ORCHIDACEAE

Corallorhiza trifida Chatelain: Rare in wet woods. (Victorin No. 794, 1906.)

**Corallorhiza maculata* Raf.: Common in rich mesic hardwoods.

**Cypripedium acaule* Ait.: Dry conifer-hardwoods. Rare.

**Cypripedium calceolus* L. *var. parviflorum* (Salisb.) Fern.: Mesic hardwoods. Rare to infrequent. (Victorin No. 11,031, 1920.)

**Epipactis helleborine* (L.) Crantz: Common in all forest types but attaining greatest occurrence in dry-mesic stands. Introduced.

**Habenaria hookeri* Torr.: Dry and dry-mesic woods. Rare.

**Habenaria hyperborea* (L.) R. Br. *var. huronensis* (Nutt.) Farw.: Wet-mesic and wet woods. Infrequent.

**Habenaria lacera* (Michx.) Lodd.: Rich, mesic shaded hardwoods. Very rare.

**Habenaria macrophylla* Goldie: Rich, mesic hardwoods. Alluvial soil. Very rare.

**Habenaria orbiculata* (Pursh) Torr.: Mesic and wet-mesic woods. Very rare.

**Habenaria psycodes* (L.) Spreng.: Wet lowland swamp conifer-hardwoods. Very rare.

**Habenaria viridis* (L.) R. Br. *var. bracteata* (Muhl.) Gray: Rich woods. (Victorin No. 785, 1906.)

**Malaxis unifolia* Michx.: Dry oak–pine woods. Very rare.

Orchis spectabilis L.: Woods on calcareous bedrock. Very rare. (Victorin No. 639, 1914.)

**Spiranthes cernua* (L.) Richard: Openings in dry-mesic hardwoods. Very rare.

**Spiranthes gracilis* (Bigel.) Beck: Openings in dry and dry-mesic woods. Very rare.

SALICACEAE

**Populus balsamifera* L.: Wet lowland swamp hardwoods and marsh edges. Very uncommon.

**Populus deltoides* Marsh.: Wet situations along south shore of Lac Hertel. Rare.

**Populus grandidentata* Michx.: Sporadic in disturbed sections of dry and dry-mesic woods. Common.

**Populus tremuloides* Michx.: Disturbed forest areas, burns and stages of secondary succession. Also in wet lowland stands. Uncommon.

**Salix bebbiana* Sarg.: Burned-over areas and damp lakeshore areas. Infrequent. (Victorin No. 271, 1913; Cleonique No. 10,379, 1938.)

**Salix discolor* Muhl.: Burned-over areas. Infrequent.

**Salix gracilis* Anderss.: Disturbed and burned-over areas and lakeshore marshes. Infrequent.

**Salix humilis* Marsh.: Dry woods and in dry disturbed or burned-over areas. Infrequent.

**Salix nigra* Marsh.: Lakeshore marshes especially in shrub or consolidation stages of hydroseric succession. Infrequent.

**Salix rigida* Muhl.: Shrub of wet thickets and marshes. Common.

MYRICACEAE

**Myrica gale* L.: Forming extensive impenetrable thickets in shallow water or recently exposed areas adjacent to Lac Hertel. Common. (Lyman, 1877.)

JUGLANDACEAE

**Carya cordiformis* (Wang.) K. Koch: Tree of dry to mesic forests, more frequent at lower altitudes on the outer slopes. Not common as mature trees.

**Carya ovata* (Mill.) K. Koch: Dry woods. Very rare, restricted to a few locations on the southwest side of the mountain.

Juglans cinerea L.: Wet-mesic and mesic hardwoods, on rich alluvial soils. Infrequent.

CORYLACEAE

**Alnus rugosa* (Du Roi) Spreng.: Wet lowland woods and swales. Restricted.

**Betula lutea* Michx. f.: One of the common deciduous trees. Attaining greatest ecological importance in wet forests.

**Betula papyrifera* Marsh.: Very common, particularly in secondary forests. Attaining greatest importance in dry and dry-mesic woods.

**Betula populifolia* Marsh.: In dry forests on steep cliffs and very disturbed situations. Very restricted.

Carpinus caroliniana Walt.: Very rare. (Victorin No. 745, 1914.)

**Corylus cornuta* Marsh.: Shrub displaying broad tolerance to moisture and occurring most frequently in wet and wet-mesic woods. Common.

Ostrya virginiana (Mill.) K. Koch: Common tree of dry woods, especially on summits. Common.

FAGACEAE

**Fagus grandifolia* Ehrh.: With *Acer saccharum* the commonest trees of the mountain. Present in all moisture segments but attaining optimum importance in dry-mesic stands.

Quercus macrocarpa Michx.: Found on the lowest terraces, adjacent to the Richelieu River but absent from the mountain proper.

**Quercus rubra* L. *var. borealis* (Michx. f.) Farw.: Very widespread in all moisture segments of the forest continuum and reaching greatest importance in dry woods, particularly on summits.

ULMACEAE

**Ulmus americana* L.: Common in wet lowland stands and attaining greatest importance in such situations.

**Ulmus rubra* Muhl.: Of infrequent occurrence but of greatest importance in wet-mesic rich hardwoods. Proliferates vegetatively forming low shrubby clumps at higher elevations.

URTICACEAE

**Laportea canadensis* (L.) Wedd.: Wet-mesic and wet hardwoods. Very common. (Lyman, 1877.)

**Pilea pumila* (L.) Gray: Wet and wet-mesic forests, particularly areas adjacent to creeks. Rare.

ARISTOLOCHIACEAE

**Asarum canadense* L.: Wet to dry-mesic woods, but reaching greatest abundance in wet-mesic rich hardwoods. Infrequent.

POLYGONACEAE

**Polygonum amphibium* L.: Floating aquatic of shallow water and exposed riparian habitats. Also in marshes. Very common.

**Polygonum arifolium* L.: Wet woods and shaded marshy situations. Rare.

**Polygonum aviculare* L.: Disturbed places, particularly roadways or wherever trampling or soil compaction occurs. Introduced.

**Polygonum cilinode* Michx.: Dry to wet-mesic woods with greatest importance in mesic woods, especially openings. Common.

**Polygonum convolvulus* L.: Introduced species of fields, orchards, and disturbed areas. Infrequent.

**Polygonum hydropiper* L.: Marshes and wet lakeshore edges. Infrequent.

**Polygonum lapathifolium* L.: Marshes. Infrequent.

**Polygonum pensylvanicum* L.: Marshes and openings in wet lowland forests. Infrequent.

**Rumex acetosella* L.: Exposed summits and areas of outcrop or shallow soils at higher altitudes. Infrequent. Introduced species.

**Rumex obtusifolius* L.: Wet lowland forest and open marshes. Infrequent introduced species. (Lyman, 1901.)

CHENOPODIACEAE

**Chenopodium album* L.: Common weed of open disturbed situations. Introduced.

**Chenopodium hybridum* L.: Introduced weed of disturbed habitats. Rare.

AMARANTHACEAE

**Amaranthus retroflexus* L.: Introduced weed of roadsides and disturbed sites. Uncommon.

AIZOACEAE

**Mollugo verticillata* L.: Wet, recently exposed gravel beaches. One location on southwest shore of Lac Hertel. Introduced.

PORTULACACEAE

**Claytonia caroliniana* Michx.: Spring herb of dry-mesic to wet-mesic hardwood forests. Most abundant in rich mesic maple–beech forest. Common. (Lyman, 1874.)

Claytonia virginica L.: Rich mesic hardwoods. Very rare.

**Portulaca oleracea* L.: Weed of wet shoreline. Very uncommon. Introduced species.

CARYOPHYLLACEAE

Arenaria lateriflora L.: Mesic hardwoods. Rare. (G. H. Du Boulay No. 548, 1949.)

**Cerastium vulgatum* L.: Introduced species of dry open disturbed habitats. Infrequent.

**Dianthus armeria* L.: Infrequent species of open grassy areas and orchards. Introduced.

Silene cucubalus Wibel.: Introduced weed. Grassy, open disturbed sites. Infrequent.

**Silene noctiflora L.*: Mesic, partly shaded disturbed areas. Infrequent introduced weed.

**Stellaria graminea* L.: Introduced weed of grassy openings and orchards. Common.

CERATOPHYLLACEAE

**Ceratophyllum demersum* L.: Submerged aquatic of shallow water. Very common in Lac Hertel.

NYMPHAEACEAE

**Nuphar variegatum* Engelm.: Floating aquatic of quiet protected waters near shore. Common in the lake.

RANUNCULACEAE

**Actaea pachypoda* Ell.: Dry-mesic, mesic, and wet-mesic forests. Infrequent.

**Actaea rubra* (Ait.) Willd.: Dry-mesic to wet forests but of greatest ecological importance in rich mesic hardwoods. Infrequent.

**Actaea rubra* (Ait.) Willd. *forma neglecta* (Gillman) Robins.: Rich mesic hardwoods. Rare.

**Anemone virginiana* L.: Restricted to dry woods. Rare or infrequent.

**Aquilegia canadensis* L.: Dry to wet-mesic woods. Greatest presence values attained in dry segment of the moisture gradient. Infrequent.

**Caltha palustris* L.: Wet lowland hardwoods and conifer-hardwoods and marshes. Infrequent.

**Clematis verticillaris* DC.: Dry oak–pine woods on rocky slopes. Rare.

Clematis virginiana L.: Dry talus slopes. Rare. (Victorin No. 8715, 1915; Cleonique No. 10,459, 1938.)

**Coptis groenlandica* (Oeder) Fern.: Wet lowland swamp conifer-hardwoods. Infrequent.

**Hepatica acutiloba* DC.: Occurring in all forest types but of greatest importance in mesic hardwoods. Infrequent.

**Hepatica americana* (DC.) Ker: Dry-mesic to wet-mesic forests, particularly dry woods. Uncommon.

Ranunculus abortivus L.: Present in dry, dry-mesic, and wet woods. Greatest abundance attained in wet situations. Infrequent.

**Ranunculus acris* L.: Grassy openings, roadsides, and orchards. Common. Introduced.
**Ranunculus flabellaris* Raf.: Wet lowland woods and lakeshores. Very rare.
**Ranunculus pensylvanicus* L. f.: Riparian marshes. Rare. (Lyman, 1877.)
**Ranunculus recurvatus* Poir.: Wet and wet-mesic woods and marshes. Infrequent.
**Ranunculus reptans* L.: Recently exposed mudflats and shorelines of the lake. Common.
**Thalictrum dioicum* L.: In all forest moisture segments but attaining maximum ecological importance in dry-mesic types. Common.
**Thalictrum polygamum* Muhl.: Wet lowland forests and marshes. Uncommon.

BERBERIDACEAE

**Caulophyllum thalictroides* (L.) Michx.: Dry-mesic to wet hardwoods. Predominant in rich mesic and wet-mesic stands. Locally abundant and generally common.
Berberis vulgaris L.: Dry-mesic forests. Very rare. Naturalized shrub.

PAPAVERACEAE

**Adlumia fungosa* (Ait.) Greene: Dry open situations, especially burned-over areas or exposed rock outcrops. Infrequent.
**Chelidonium majus* L.: Disturbed mesic hardwoods. Infrequent. Introduced.
**Corydalis sempervirens* (L.) Pers.: Open rocky summits, exposed outcrops, dry and burned-over dry woods. Common. (Lyman, 1877.)
**Dicentra cucullaria* (L.) Bernh.: Spring plant of rich mesic hardwoods. Common.
**Dicentra canadensis* (Goldie) Walp.: Common vernal of rich mesic hardwoods.
**Sanguinaria canadensis* L.: Dry-mesic to wet-mesic hardwoods. Optimum frequency in mesic hardwoods. Common.

CRUCIFERAE

**Arabis divaricarpa* Nels.: Dry woods and shaded outcrops. Common.
Arabis drummondi Gray: Dry woods. Rare. (Cleonique No. 10,460, 1938.)
Arabis hirsuta (L.) Scop.: Dry woods and outcrops. Rare. (Cleonique No. 10,483, 1938.)
**Arabis laevigata* (Muhl.) Poir.: Dry to mesic woods often on outcrop sites or on ledges. Common.
**Barbarea vulgaris* R. Br.: Burned-over woods and roadsides. Infrequent.
**Capsella bursa-pastoris* (L.) Medic.: Weed of roadsides and other disturbed sites. Common. Introduced.
**Cardamine parviflora* L. *var. arenicola* (Britt.) O. E. Schulz: Dry exposed rocky situations on the higher summits. Rare.
**Cardamine pensylvanica* Muhl.: Wet woods, marshes, and exposed shore flats. Common.
**Dentaria diphylla* Michx.: Occurrence restricted to dry-mesic hardwoods. Infrequent.
Draba arabisans Michx.: Rocky outcrops of the highest summits. Rare. (Victorin No. 3867, 1912.)
**Rorippa islandica* (Oeder) Borbas: Marshes and muddy lakeshore areas. Common.
**Thlaspi arvense* L.: Introduced weed of disturbed areas. Infrequent.

SAXIFRAGACEAE

**Chrysosplenium americanum* Schwein.: Wet swamp woods and wet woodland depressions. Infrequent.
**Mitella diphylla* L.: Dry-mesic to wet woods. Optimum occurrence in mesic woods. Common.
**Mitella nuda* L.: Abundant in lowland conifer-hardwoods.
**Penthorum sedoides* L.: Marshes and riparian habitats. Rare.
Ribes americanum Mill.: Dry woods. Rare or infrequent.
**Ribes cynosbati* L.: Present throughout the forest moisture continuum but reaching optimum importance in dry-mesic and mesic hardwoods. Common.
**Ribes glandulosum* Grauer: Mesic to wet forests. Common.
**Ribes lacustre* (Pers.) Poir.: Wet to mesic forests; most frequent in wet lowland types. Infrequent.
**Ribes triste* Pall.: Wet swamp hardwoods and wet-mesic woods. Common.

**Saxifraga virginiensis* Michx.: Dry and dry-mesic woods on rigorous slopes. Abundant on rock ledges and outcrops but otherwise infrequent.

**Tiarella cordifolia* L.: Wet to dry-mesic woods. Abundant, particularly in mesic hardwoods.

HAMAMELIDACEAE

**Hamamelis virginiana* L.: Wet-mesic conifer-hardwoods on steep slopes. Very rare.

ROSACEAE

**Agrimonia gryposepala* Wallr.: Wet lowland woods and openings. Infrequent.

Amelanchier arborea (Michx. f.) Fern.: Infrequent. (Cinq-Mars, 1958; Cleonique No. 9541, 1937.)

**Amelanchier bartramiana* (Tausch) Roemer: Dry woods, particularly on summit situations. Infrequent.

**Amelanchier laevis* Wieg.: Dry conifer-hardwoods. Infrequent. (Victorin No. 5324, 1913.)

Amelanchier sanguinea (Pursh) DC.: Woods at lower altitudes. (Cleonique No. 10,284, 1938; Cinq-Mars, 1958.)

Amelanchier stolonifera Wieg.: Open rocky summits. Common. (Victorin No. 11,218, 1920; Cleonique No. 9951, 1938.)

Crataegus brainerdi Sarg.: Thickets at lower altitudes. (Cinq-Mars, 1958.)

**Crataegus jackii* Sarg.: Dry open thickets on outcrops on the higher summits. (Cleonique No. 9513, 1937.)

Crataegus pruinosa (Wendl.) K. Koch: Thickets and open woods at lower levels (Cinq-Mars, 1958.)

Crataegus punctata Jacq.: Thickets at lower levels. (Victorin No. 11,194, 1920.)

Crataegus submollis Sarg.: Thickets. (Cinq-Mars, 1958.)

**Fragaria vesca* L.: Dry woods on steep slopes. Rare.

**Fragaria virginiana* Duchesne: Species of broad ecological tolerance for moisture conditions, but occurring with highest frequency in dry and wet woods. Common.

**Geum canadense* Jacq.: Wet and wet-mesic woods. Also frequent in wet openings. Common.

**Geum rivale* L.: Wet and wet-mesic woods. Infrequent.

**Potentilla anserina* L.: Gravel–sand beaches, south shore of Lac Hertel. Common.

Potentilla argentea L.: Introduced weed of roadsides. Infrequent.

**Potentilla arguta* Pursh: Dry open grassy summit balds. Rare. (Victorin No. 740, 1914; Cinq-Mars, 1954.)

Potentilla norvegica L.: Introduced species of roadsides and orchards. Common. (Lyman, 1877.)

Potentilla palustris (L.) Scop.: Wet marshes and marsh fringes in shallow water. Rare.

**Potentilla tridentata* Ait.: Rocky exposed summit of the Sugar Loaf. Rare. (Victorin No. 681, 1905; Mignault, 1878.)

**Prunus pensylvanica* L. f.: Dry to wet-mesic secondary woods or areas of local disturbance within such woods. Common.

**Prunus serotina* Ehrh.: Occurring throughout the forest moisture continuum. Highest importance in mesic woods. Uncommon.

**Prunus virginiana* L.: Commonest shrub of the mountain. Exhibiting broad ecological tolerance for almost all habitats except marshes. Greatest frequencies attained in dry-mesic and mesic forests. Forms extensive thickets on the open higher summits.

**Pyrus decora* (Sarg.) Hyland: Mesic forests and openings on steep rocky hillsides at higher elevations. Infrequent.

**Pyrus melanocarpa* (Michx.) Willd.: Dry burned-over areas. Infrequent. (Cleonique No. 10,438, 1938.)

Rosa carolina L. (?): Dry woods and thickets. Common.

Rosa nitida Willd.: Wet lowland forests and lakeshore margin. Infrequent.

Rosa sp.: Woods and thickets. Common.

**Rubus alleghieniensis* Porter (?): Dry to wet-mesic woods. Prevalent in dry-mesic woods and locally abundant in burned areas. Common.

Rubus idaeus L.: Wet to mesic woods, in areas of local disturbance and on the shrubby fringes of marshes as well as burned areas. Infrequent.

Rubus occidentalis L.: Woods of the dry end of the moisture series. Infrequent. (Victorin No. 711, 1914.)

**Rubus odoratus* L.: Wet-mesic to dry woods and clearings at lower levels. Common.

**Rubus pubescens* Raf.: Wet lowland to dry woods. Attaining greatest frequency in wet conifer-hardwoods. Common.

**Spiraea alba* Du Roi: Burned-over areas. Rare.

**Spiraea latifolia* (Ait.) Borkh.: Wet lowland forest, marshes, and damp shores. Occasionally found on summit balds. Infrequent.

**Spiraea tomentosa* L.: Wet forests, marshes, and low ground. Rare.

Waldsteinia fragarioides (Michx.) Tratt.: Dry conifer-hardwoods and pine groves. Rare.

LEGUMINOSAE

**Amphicarpa bracteata* (L.) Fern.: Of broad ecological tolerance and growing in forests throughout the moisture continuum. Of optimum importance in wet-mesic and dry types. Common.

**Desmodium canadense* (L.) DC.: Damp openings. Rare.

**Desmodium glutinosum* (Muhl.) Wood: Dry and dry-mesic woods and occasionally in wet woods. Common.

**Trifolium agrarium* L.: Fields and orchard. Common. Introduced.

**Trifolium hybridum* L.: Introduced species of fields and orchard. Common.

**Trifolium pratense* L.: Fields and orchard. Common. Introduced.

**Trifolium repens* L.: Fields, orchard, and open roadsides. Common. Introduced.

**Vicia cracca* L.: Orchard and open fields. Very common introduced species.

OXALIDACEAE

**Oxalis europaea* Jord.: Damp, shaded openings. Rare.

**Oxalis montana* Raf.: Wet and wet-mesic conifer-hardwoods.

GERANIACEAE

**Geranium robertianum* L.: Dry and dry-mesic woods, especially pine groves. Rare. (Lyman, 1877; Victorin No. 766, 1914.)

EUPHORBIACEAE

**Acalypha rhomboidea* Raf.: Wet lakeshores. Infrequent.

ANACARDIACEAE

**Rhus radicans* L.: Dry and dry-mesic woods on steep slopes and rocky talus at lower elevations. Infrequent.

**Rhus typhina* L.: Dry forests and dry openings or opened slopes. Rare.

AQUIFOLIACEAE

**Ilex verticillata* (L.) Gray: Shrub stage of marsh succession. Rare.

CELASTRACEAE

**Celastrus scandens* L.: Dry-mesic and dry hardwoods, particularly on open rocky slopes. Infrequent.

STAPHYLEACEAE

**Staphylea trifolia* L.: Mesic hardwoods at lower elevations. Very rare.

ACERACEAE

**Acer pensylvanicum* L.: Present in all segments of the forest moisture series but reaching highest importance values in mesic and wet types. Common. (Lyman, 1877.)

**Acer rubrum* L.: Predominant in wet and wet-mesic stands. Highest importance in wet swamp forests. Infrequent.

**Acer saccharum* Marsh.: Commonest tree of the mountain and responsible for a great proportion of the forest cover. Occurring in all moisture segments and with greatest importance in mesic, dry-mesic, and wet-mesic stands, in that order.

**Acer spicatum* Lam.: Seldom attaining tree size, but widespread in wet to dry-mesic stands and forming a tall shrub layer. Greatest importance in mesic and wet forest types.

BALSAMINACEAE

**Impatiens capensis* Meerb.: Wet and wet-mesic hardwood forest and in partially shaded wet openings along streams. Common.

**Impatiens pallida* Nutt.: Very infrequent. Confined to mesic hardwood forests on rich soils.

VITACEAE

**Parthenocissus quinquefolia* (L.) Planch.: Wet, dry-mesic, and dry forests. Exhibiting a peculiar tolerance for the extremes of moisture and yet absent from intermediate moisture sites. Infrequent.

**Vitis riparia* Michx.: South shore of Lac Hertel. Rare.

TILIACEAE

**Tilia americana* L.: Found in all segments of the moisture continuum, predominant in mesic, wet-mesic, and dry-mesic types. Common.

MALVACEAE

**Malva moschata* L.: Introduced species of open fields of the terraces of the mountain. Infrequent.

GUTTIFERAE

**Hypericum majus* (Gray) Britt.: Wet marshes adjacent to lakeshores. Rare.

**Hypericum mutilum* L.: Wet lowland woods and marshes. Rare.

Hypericum perforatum L.: Introduced weed of open disturbed fields. Uncommon.

**Hypericum virginicum* L.: Marshes and wet shorelines. Infrequent.

VIOLACEAE

**Viola canadensis* L.: Dry to wet-mesic deciduous woods. Most frequent in mesic types. Common. (Lyman, 1877.)

Viola conspersa Reichenb. (Victorin No. 11,406, 1920.)

**Viola cucullata* Ait.: Dry-mesic and mesic hardwood forests. Rare. (Lyman, 1891.)

**Viola incognita* Brainerd: Wet to mesic forests. Highest frequency attained in wet-mesic situations. Common.

Viola pallens (Banks) Brainerd: Wet woods. Uncommon.

**Viola papilionacea* Pursh: Dry woods. Infrequent.

**Viola pensylvanica* Michx.: Rich wet-mesic forests. Rare.

**Viola pubescens* Ait.: Widely distributed in all forest types. Most frequently occurring in wet-mesic, mesic, and dry-mesic sites. Very common.

**Viola renifolia* Gray: Species of broad ecological tolerance, attaining optimum frequency in wet-mesic woods. Common.

**Viola selkirkii* Pursh: Dry-mesic, mesic, and wet-mesic forests. Common. (Campbell, 1886.)

**Viola septentrionalis* Greene: Rich wet-mesic woods. Rare. (Cinq-Mars, 1954.)

**Viola sororia* Willd.: Dry to wet-mesic forests. Infrequent.

THYMELAEACEAE

**Dirca palustris* L.: Common shrub of dry to wet-mesic forests attaining maximum frequency in dry-mesic and mesic stands. Common.

LYTHRACEAE

**Lythrum salicaria* L.: Marshes and wet shores. Introduced species. Infrequent. (Lyman, 1901.)

ONAGRACEAE

**Circaea alpina* L.: Wet to mesic woods, particularly near streams. Infrequent.

**Circaea quadrisulcata* (Maxim.) Franch. & Sav. *var. canadensis* (L.) Hara: In woods throughout the moisture continuum but reaching optimum frequency in wet-mesic and wet forest stands. Common. (Lyman, 1877.)

**Epilobium angustifolium* L.: Dry woods and disturbed or burned situations. Infrequent.

**Epilobium coloratum* Biehler: Marshes and open lakeshore habitats. Rare.

**Epilobium glandulosum* Lehm.: Wet and wet-mesic lowland forests. Uncommon.

**Epilobium glandulosum* Lehm. *var. adenocaulon* (Haussk.) Fern.: Dry scrub oak woods at higher elevations. Rare.

**Ludwigia palustris* (L.) Ell. *var. americana* (DC.) Fern. & Grisc.: Lakeshore marshes. Infrequent.

**Oenothera biennis* L.: A weedy species frequent in dry openings and less frequent in dry woods. Common.

**Oenothera perennis* L.: Open grassy fields. Rare.

ARALIACEAE

Aralia hispida Vent.: Dry burned-over woods. Very rare.

**Aralia nudicaulis* L.: Very ubiquitous species recorded present in many stands in all segments of the moisture gradient. Common.

**Aralia racemosa* L.: Dry-mesic, mesic, and wet-mesic woods. Often encountered along trails in these forest types. Frequent.

**Panax quinquefolius* L.: Dry-mesic to wet woods. Most frequent in wet-mesic woods. Rare.

Panax trifolius L.: Wet lowland woods. Infrequent.

UMBELLIFERAE

Cicuta bulbifera L.: Wet woods and marshes. Rare.

**Cicuta maculata* L.: Wet woods and marshes. Infrequent.

**Cryptotaenia canadensis* (L.) DC.: Wet-mesic, wet, and dry-mesic hardwoods. Common.

**Hydrocotyle americana* L.: Wet lowland woods. Common.

**Osmorhiza claytoni* (Michx.) C. B. Clarke: Very common in all types of woods. Attaining greatest frequency in mesic types.

**Sanicula gregaria* Bickn.: Dry-mesic and wet-mesic hardwoods. Infrequent.

**Sanicula marilandica* L.: Wet to dry woods. Most common in wet-mesic types. (Lyman, 1877.)

**Sanicula trifoliata* Bickn.: Wet-mesic and wet forest types. Often found along moist shaded paths. Infrequent.

**Sium suave* Walt: Wet lowland woods and open marshes. Infrequent.

**Cornus alternifolia* L. f.: Dry-mesic to wet woods. Most frequent in wet-mesic and mesic types. Common.

**Cornus canadensis* L.: Species of bimodal tolerance found in dry forests and wet forests but absent from intermediate situations. Rare.

**Cornus rugosa* Lam.: Dry and dry-mesic woods. Infrequent.

**Cornus stolonifera* Michx.: Wet lowland forests, marshes, and wet lakeshores. Infrequent.

PYROLACEAE

**Chimaphila umbellata* (L.) Bart. *var. cisatlantica* Blake: Dry and dry-mesic forest types. Rare.

**Monotropa hypopithys* L.: Mesic to wet woods. Very rare.

**Monotropa uniflora* L.: Present in all segments of the forest moisture series. Most frequent in dry-mesic stands. Common and abundant on the mountain.

Pyrola asarifolia Michx.: Dry and dry-mesic stands. Rare.

**Pyrola elliptica* Nutt.: Common in all segments of the forest moisture continuum. Most frequent in wet stands. (Lyman, 1901.)

**Pyrola secunda* L.: Dry to wet-mesic woods, occurring most frequently in dry types. Common.

**Pyrola virens* Schweigger: Dry woods. Rare.

ERICACEAE

**Arctostaphylos uva-ursi* (L.) Spreng.: Dry woods and burned-over dry forest. Very rare. (Victorin No. 746, 1914.)

**Gaultheria procumbens* L.: Infrequent in dry forests.

Gaylussacia baccata (Wang.) K. Koch: Dry woods. Very rare.

**Kalmia angustifolia* L.: Very rare in dry woods or openings in dry woods.

Vaccinium angustifolium Ait.: Infrequent in dry woods, especially coniferous or coniferous–deciduous types.

**Vaccinium corymbosum* L.: Damp lakeshores. Very rare.

**Vaccinium myrtilloides* Michx. Common in dry and dry-mesic woods, particularly open pine woods. (Lyman, 1877.)

PRIMULACEAE

**Lysimachia ciliata* L.: Common in wet lowland woods.

**Lysimachia clethroides* Duly: Burned-over dry woods. Uncommon garden escape.

**Lysimachia hybrida* Michx.: Marshes. Very rare. (Lyman, 1877.)

**Lysimachia terrestris* (L.) BSP.: Wet lowland forests and open marshes. Common.

**Lysimachia thyrsiflora* L.: Wet lakeshore marshes. Very rare.

**Trientalis borealis* Raf.: Abundant in all forest types, particularly wet and mesic conifer-hardwoods.

OLEACEAE

**Fraxinus americana* L.: In all forest types but attaining optimum importance in wet-mesic stands. Also noticeable in dry and dry-mesic summit forests.

**Fraxinus nigra* Marsh.: Wet and wet-mesic lowland stands. Rare.

GENTIANACEAE

**Menyanthes trifoliata* L. *var. minor* Raf.: Wet, open woodland depressions. Very rare.

APOCYNACEAE

**Apocynum androsaemifolium* L.: Dry to wet-mesic woods, but absent in the mesic segment of the forest moisture series. Attaining greatest frequency in dry woods, open grassy meadows, fields, and burned-over forests. Common.

ASCLEPIADACEAE

**Asclepias syriaca* L.: Orchards, grassy openings. Common.

CONVOLVULACEAE

**Convolvulus sepium* L.: Orchards and fields. Uncommon.

HYDROPHYLLACEAE

**Hydrophyllum virginianum* L.: Rich mesic and dry-mesic hardwoods. Rare.

BORAGINACEAE

**Hackelia americana* (Gray) Fern.: Dry openings, often on hornfels, in hardwoods, at lower elevations. Rare.

**Symphytum officinale* L.: Introduced species. (Lyman, 1884.)

VERBENACEAE

**Verbena hastata* L.: Wet woods along lakeshore. Rare.

LABIATAE

**Galeopsis tetrahit* L.: Wet-mesic hardwoods. Infrequent.

**Lycopus americanus* Muhl.: Wet lowland hardwood-conifer woods. Infrequent.

**Lycopus uniflorus* Michx.: Wet lowland woods and marshes. Common.

**Mentha arvensis* L.: Wet woods, marshes, and lakeshores. Infrequent.

**Prunella vulgaris* L.: Wet woods and wet to mesic forest openings. Common.

**Prunella vulgaris* L. *forma albiflora* (Bogenhard) Britt.: Openings in mesic woods. Rare.

**Satureja vulgaris* (L.) Fritsch. *var. neogaea* Fern.: Dry woods and grassy balds. Infrequent.

**Scutellaria epilobiifolia* A. Hamilton: Marshes. Common.

**Scutellaria lateriflora* L.: Wet and wet-mesic lowland woods and marshes. Common. (Lyman, 1877.)

SOLANACEAE

**Solanum americanum* Mill.: Burned-over woods and clearings. Rare.

SCROPHULARIACEAE

**Chelone glabra* L.: Wet lowland woods. Common.

**Gratiola virginiana* L.: Marshes and exposed riparian habitats. Infrequent.

**Linaria vulgaris* Hill: Introduced weed of grassy fields, orchards, and burned-over forests. Common.

**Mimulus ringens* L.: Wet lowland swamps and marshes. Rare.

**Pedicularis canadensis* L.: Wet woods and dry pine groves. Infrequent.

Scrophularia lanceolata Pursh: Shaded openings and borders of woods. Rare.

**Verbascum thapsus* L.: Introduced weed of disturbed places and orchards. Infrequent.

**Veronica americana* (Raf.) Schwein.: Shoreline marshes. Rare.

**Veronica officinalis* L.: Pine groves and dry grassy openings. Common.

**Veronica scutellata* L.: Shoreline marshes. Common. (Lyman, 1877.)

**Veronica serpyllifolia* L.: Damp shaded openings and marshes. Introduced species. Infrequent.

OROBANCHACEAE

**Epifagus virginiana* (L.) Bart.: Dry-mesic to wet woods with optimum occurrence in wet-mesic types. Common but restricted to forest types containing *Fagus*.

PHRYMACEAE

Phryma leptostachya L.: Hardwoods. Rare.

PLANTAGINACEAE

**Plantago major* L.: Introduced weed of roadsides and disturbed areas. Uncommon.

RUBIACEAE

**Galium asprellum* Michx.: Wet lowland woods and marshes. Infrequent.

**Galium boreale* L.: Open grassy fields and orchards. Infrequent.

Galium circaezans Michx. *var. hypomalacum* Fern.: Dry-mesic and mesic hardwoods. Rare.

**Galium labradoricum* Wieg.: Wet swamps and marshes. Common.

**Galium lanceolatum* Torr.: Dry to mesic woods. Common. (Victorin No. 757, 1905; Blackader, 1882.)

**Galium obtusum* Bigel.: Wet and wet-mesic woods and marshes. Common.

**Galium palustre* L.: Infrequent in marshes. (Lyman, 1877.)

**Galium triflorum* Michx.: Common throughout the forest moisture continuum but reaching optimum occurrence in wetter segments of the gradient.

**Mitchella repens* L.: Common in all moisture segments of the forest gradient and occurring most frequently in the wet-mesic types. (Lyman, 1901.)

CAPRIFOLIACEAE

**Diervilla lonicera* Mill.: Dry, dry-mesic, and mesic woods. Particularly abundant in dry openings on the higher summits.

**Linnaea borealis* L. *var. americana* (Forbes) Rehd.: Wet lowland and dry upland coniferous hardwoods. A species apparently existing under extremes of moisture conditions. Rare.

**Lonicera canadensis* Bartr.: Species of broad ecological tolerance, occurring with high frequency in all moisture situations and in all forest types of the mountain. Common.

**Lonicera dioica* L.: Dry woods. Infrequent.

**Sambucus canadensis* L.: Wet and wet-mesic woods and wet clearings. Common.

**Sambucus pubens* Michx.: Predominantly in mesic woods but also attaining high values in dry-mesic, wet-mesic, and dry forest situations.

**Symphoricarpos albus* (L.) Blake: Rare in dry woods.

**Triosteum aurantiacum* Bickn.: Dry woods and dry pine groves. Very rare.

**Viburnum acerifolium* L.: Dry to mesic woods but occurring most frequently in dry types. Common. (Lyman, 1877.)

**Viburnum alnifolium* Marsh.: Most frequently in wet-mesic stands but encountered throughout the entire forest moisture continuum. Common. (Lyman, 1877.)

Viburnum cassinoides L.: Wet woods. Rare. (Victorin, 1912.)

**Viburnum trilobum* Marsh.: Wet and wet-mesic lowland woods, especially near streams. Rare.

CAMPANULACEAE

**Campanula rotundifolia* L.: Very rare. Rocky crevices and outcrop on the higher summits.

**Lobelia cardinalis* L.: Rare. Wet woods and lakeshore marshes. (Lyman, 1877.)

**Lobelia inflata* L.: Dry-mesic and mesic hardwoods, often on the edges of paths. Common.

Lobelia spicata Lam.: Mesic openings. Very rare.

COMPOSITAE

**Achillea millefolium* L.: Dry woods and as a weed in dry disturbed areas or openings. Common.

**Ambrosia artemisiifolia* L.: Weed of disturbed situations and roadsides. Common.

**Anaphalis margaritacea* (L.) C. B. Clarke: Dry woods, openings, and clearings. Often associated with rock outcrop. Infrequent.

Antennaria canadensis Greene: Dry woods and openings in dry woods. Infrequent. (Du Boulay No. 553, 1949.)

Antennaria fallax Greene: Dry woods. Rare. (Victorin No. 5536, 1914; Cleonique No. 10,481, 1938.)

**Antennaria munda* Fern.: Dry woods. Infrequent.

**Antennaria neglecta* Greene: Dry woods on hilltops. Infrequent.

**Antennaria neodioica* Greene: Dry woods on rocky slopes. Common.

**Antennaria plantaginifolia* (L.) Hook.: Dry woods at higher elevations. Infrequent.

**Arctium minus* (Hill) Bernh.: Introduced weed of disturbed roadsides and clearings. Infrequent.

**Aster acuminatus* Michx.: Attaining high frequency values in all forests types on the mountain. Very common.

Aster ciliolatus Lindl.: Mesic hardwood forests. Infrequent.

**Aster cordifolius* L.: A species of broad ecological tolerance for all forest moisture situations. Predominating in dry-mesic and wet-mesic types. Common.

Aster lateriflorus (L.) Britt.: Wet lowland woods and marshes. Infrequent.

**Aster macrophyllus* L.: Of broad ecological tolerance but most frequent in dry-mesic and dry woods. Common.

**Aster novae-angliae* L.: Dry woods, particularly scrub oak–maple types. Rare.

**Aster ontarionis* Wieg.: Infrequent in low-lying open or partially shaded areas adjacent to the lake.

**Aster puniceus* L.: Wet and wet-mesic woods. Infrequent.

**Aster simplex* Willd.: Disturbed fields, clearings, and burned-over areas. Infrequent.

**Aster umbellatus* Mill.: Infrequent in wet-mesic woods.

**Bidens cernua* L.: Marshes and recently exposed riparian situations. Common.

**Bidens frondosa* L.: Wet and wet-mesic lowland woods, marshes, and other lakeshore habitats. Common.

**Chrysanthemum leucanthemum* L.: Fields and disturbed areas. Common also in orchards. Introduced.

**Cichorium intybus* L.: Introduced weed of disturbed fields and orchards. Common.

Cirsium arvense (L.) Scop.: Damp situations along the lakeshore. Very uncommon. Introduced.

**Erechtites hieracifolia* (L.) Raf.: Open marshes, lakeshores, and burned-over woods. Infrequent.

**Erigeron annuus* (L.) Pers.: Plant of open disturbed habitats, particularly fields and outcrops. Infrequent.

**Erigeron canadensis* L.: Weed of roadsides. Common.

**Erigeron philadelphicus* L.: Dry oak-pine woods. Infrequent.

**Erigeron strigosus* Muhl.: Open fields and orchards. Common.

**Eupatorium maculatum* L.: Wet lowland woods and marshes. Common.

Eupatorium perfoliatum L.: Wet woods, marshes, and damp openings. Common.

**Eupatorium rugosum* Houtt.: Wet-mesic to dry hardwoods. Often growing along pathways in these woods. Common.

**Galinsoga ciliata* (Raf.) Blake: Introduced weed of damp, shaded, disturbed situations. Common.

**Gnaphalium uliginosum* L.: Exposed mud flats along lakeshore and gravel–sand beaches. Common in these habitats.

**Hieracium aurantiacum* L.: Disturbed places, fields, and orchards. Common. Introduced.

Hieracium canadense Michx.: Dry, open conifer-hardwoods and openings in this forest type. Infrequent.

**Hieracium florentinum* All.: Introduced species of disturbed sites in dry-mesic conifer-hardwoods. Rare.

**Hieracium paniculatum* L.: Dry-mesic and wet-mesic woods. Infrequent.

**Hieracium scabrum* Michx.: Infrequent in forest types at the dry end of the moisture continuum.

**Lactuca biennis* (Moench) Fern.: Weedy species of fields and openings. Common.

**Prenanthes alba* L.: Widespread species, in all forest types, attaining optimum occurrence in wet woods.

**Prenanthes altissima* L.: Infrequent species in lowland swamp forests.

**Rudbeckia serotina* Nutt.: Disturbed fields and orchards. Common. Introduced.

**Solidago canadensis* L.: Openings, fields, and burned-over forests. Common.

**Solidago caesia* L.: Species of broad ecological tolerance to forest moisture conditions, present in all forest moisture segments. Common in all types but of most frequent occurrence in dry to mesic segments.

**Solidago flexicaulis* L.: Species of broad ecological tolerance occurring throughout the forest moisture continuum but predominating in wet-mesic types. Common.

**Solidago graminifolia* (L.) Salisb.: In fields and along lakeshore. Common.

**Solidago hispida* Muhl.: Dry and dry-mesic woods and outcrop openings in dry forest. Infrequent.

**Solidago juncea* Ait.: Dry to wet-mesic woods. Common.

**Solidago rugosa* Ait.: Dry scrubby woods. Infrequent.

**Solidago squarrosa* Muhl.: Dry woods, particularly conifer-hardwoods, and openings in these woods.

**Sonchus arvensis* L.: Weed of open disturbed places. Introduced. Common.

**Sonchus asper* (L.) Hill: Introduced species of disturbed areas. Common.

**Sonchus oleraceus* L.: Introduced species of fields, orchards, and other disturbed situations. Common.

**Tanacetum vulgare* L.: Introduced species, common in orchards.

**Taraxacum officinale* Weber: Infrequent, introduced weed of disturbed areas.

**Tragopogon pratensis* L.: Introduced species of fields and orchards. Uncommon.

THE PHYSIOLOGY OF HOST–PARASITE RELATIONS

VIII. EFFECTS OF RUST INFECTION ON ASCORBIC ACID AND GLUTATHIONE IN WHEAT LEAVES[1]

B. I. SAHAI[2] AND MICHAEL SHAW

Abstract

First seedling leaves of Little Club (susceptible), Khapli I (moderately susceptible), and Khapli (resistant) wheats were inoculated with *Puccinia graminis tritici* Erikss. and Henn. (Race 15B). Ascorbic (AA) and dehydroascorbic (DHA) acids and glutathione (GSH) were measured at 1–2 day intervals after inoculation until after sporulation. After infection, AA and DHA increased in Little Club and decreased in Khapli. In Khapli the DHA/AA ratio rose sharply 6–8 days after inoculation; in Little Club a similar rise occurred 15–16 days after inoculation. In Khapli I and Little Club, but not in Khapli, GSH increased sharply when sporulation occurred. AA was not detected in uredospores, but DHA was. Oxidized glutathione was not detected in healthy or infected leaves but was present in uredospores. The results are discussed briefly in relation to the changes in auxin content and redox potentials caused by rust infection.

Introduction

Recent work suggests that the roles of auxin, ascorbic acid, and glutathione may be intimately interconnected. Thus Chinoy *et al.* (1957) have demonstrated a statistically significant interaction between ascorbic acid and IAA and Marré's group (see Galston and Purves 1960) have concluded that auxin promotes the formation of the reduced forms of glutathione and ascorbate. Infection with rust has been shown to raise the auxin content of susceptible hosts (Shaw and Hawkins 1958; Sahai 1960). However, Pilgrim and Futrell (1957) reported that there was no consistent relationship between the ascorbic acid content of uninfected plants and resistance or susceptibility to rust and also that infection had only slight effects on the ascorbic acid levels in several varieties of wheat.

In this laboratory, infection with stem rust has been found to have well-defined, characteristic effects on the levels of ascorbic acid and glutathione in the first seedling leaves of Little Club and Khapli wheats. The results of a series of analyses of infected and uninfected leaves and of uredospores of *P. graminis tritici* are presented in this paper.

Plant Material

Little Club (rust reaction type 4), Khapli I (rust reaction type 2–3), and Khapli (rust reaction type 1) wheats were grown in the greenhouse and the first leaves were heavily inoculated with race 15B of *P. graminis tritici* Erikss. and Henn. Khapli I is an off-type Khapli grown in the field plots of the Department of Biology. Growing conditions and methods of inoculation have already been described (Samborski and Shaw 1956).

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Uredospores of race 15B were cultured on Atlas 46 barley. They were germinated overnight in darkness in enamel trays and then collected and washed on filter paper by suction using a Büchner funnel. Some bacteria inevitably develop when spores are germinated in this way, but this method of collecting the spore material removes most of them.

Methods

Ascorbic (AA) and Dehydroascorbic (DHA) Acids

These were determined by Bessey's method (1938) with slight modifications. Leaves (1–3 g) or uredospores (200–600 mg) were ground with cold 4% metaphosphoric acid in a high-speed blendor for 3 minutes. In some analyses the uredospores were ground in a ball mill with 4% metaphosphoric acid saturated with nitrogen for 2 hours at 0° C. Whatever the procedure, the extract was quantitatively transferred to a volumetric flask with repeated rinsings with 4% metaphosphoric acid and then filtered. The first 10 ml of filtrate was discarded. The next 25 ml was mixed with 7 ml of citrate buffer (21 g citric acid and 200 ml 1*N* NaOH brought to 250 ml). The final pH of the mixture was 3.5 to 3.6.

One-milliliter portions of the buffered extract were pipetted into three matched cuvettes. Distilled water (2.5 ml) was added to one cuvette for which the spectrophotometer (Beckman, Model DU) was adjusted to read zero density at 520 mμ. Standardized 2,6-dichlorophenol–indophenol solution (2.5 ml) was added from a fast-delivery pipette to the other two cuvettes. The decrease in optical density caused by reduction of the dye was determined from readings taken 15 seconds later. Dye solutions were standardized by mixing 2.5 ml of dye (10 to 13 mg/liter) with 1 ml of buffer (25 parts 4% HPO_3 and 7 parts citrate buffer) and reading the optical density against distilled water in the spectrophotometer. The method was calibrated using known concentrations of ascorbic acid. The value of the instrument-constant (Evelyn *et al.* 1938) so calculated using different concentrations of ascorbic acid on different occasions over a period of nearly a year was found to have a standard deviation of about 4%.

Dehydroascorbic acid was determined from the difference between the values for ascorbic acid before and after reduction of the extract with H_2S. H_2S was bubbled through about 10 ml of buffered extract for 15 minutes. The extract was then allowed to stand for 2 hours at room temperature after which the H_2S was removed by bubbling with wet nitrogen for 1 hour. Total ascorbic acid was then determined as described above. Buffer treated with H_2S in the manner described did not reduce the dye, indicating that the removal of excess gas was satisfactory.

Reduced Glutathione (GSH)

This was determined by the glyoxylase method of Patterson and Lazarow (1955), with slight modifications. Oxidized glutathione (GSSG) was measured after reduction with cysteine (Racker 1951).

Leaves (2–4 g) or uredospores (1 g) were ground in 6–8 ml of cold sulphosalicylic acid with a mortar and pestle, allowed to stand for 30 minutes, ground again, and centrifuged. The pH of the supernatant was adjusted to 4.5 (glass

electrode) with 0.2*M* sodium bicarbonate. Estimation of glutathione was then carried out in the Warburg manometric apparatus. To the main chamber of each flask were added 0.8 ml of a 20% suspension of acetone-dried yeast, 0.3 ml methyl-glyoxal, and 0.4 ml 0.2*M* sodium bicarbonate. Extract was placed in the side arm of each flask. Three additional flasks were always included in each run. One served as a thermobarometer. The side arms of the other two contained, respectively, 0.5 ml of standard glutathione solution (10 mg/100 ml) dissolved in 2% sulphosalicylic acid previously adjusted to pH 4.5 with sodium bicarbonate, and 0.5 ml of the bicarbonate–sulphosalicylic acid mixture alone.

After 20 minutes' equilibration at 25±0.02° C the flasks were tipped and shaking continued for 5 minutes with the stopcocks open. The stopcocks were then closed and readings taken at 5-minute intervals for 25 minutes. The volume of carbon dioxide produced in the last 20 minutes was used in the calculations, a correction being made for the volume of gas liberated in the absence of plant extract or glutathione. The curve for CO_2 production vs. glutathione (2.5–20 mg/100 ml) was both linear and reproducible. Nevertheless the amount of glutathione in the unknown samples was always determined by calculation from the corrected volume of CO_2 produced in 20 minutes and the corrected volume of CO_2 produced by the glutathione standard measured in each experiment.

Determinations of both ascorbic acid and glutathione were made at the same time for both infected and uninfected first seedling leaves. Parallel samples were collected for measurements of dry weight (Samborski and Shaw 1956).

Results

Analyses for ascorbic and dehydroascorbic acids and reduced and oxidized glutathione were carried out at intervals over a period of about a year. The results for uninfected wheat leaves and uredospores are summarized in Table I. There was no difference between the levels of ascorbic acid in Khapli and Little Club or between the levels of reduced glutathione in Little Club and Khapli I.

Surprisingly, ascorbic acid was not detected in uredospores although appreciable amounts of dehydroascorbate were present. Ascorbic acid may be

TABLE I

Ascorbic and dehydroascorbic acids and glutathione in uninfected wheat leaves and uredospores*

Plant material	AA, mg/100 g fresh weight	DHA, mg/100 g fresh weight	GSH, mg/100 g fresh wt.	GSSG, mg/100 g fresh wt.
Little Club leaves	38.0±8.2† (n=19)	7.4±2.4† (n=18)	7.68±2.0 (n=14)	Not detected
Khapli leaves	35.0±9.1 (n=17)	4.63±1.3 (n=17)	3.97±0.4 (n=5)	Not detected
Khapli I leaves	Not analyzed	Not analyzed	8.54±1.0 (n=10)	Not detected
Uredospores (ungerminated)	Not detected	30.7±10.3 (n=6)	29.4 (n=2)	6.2 (n=2)
Uredospores (germinated)	Not detected	16.8±2.6 (n=4)	Not analyzed	Not analyzed

*Dry wt. of leaves =10–12% of fresh wt.
Dry wt. of uredospores =approx. 80% of fresh wt.
†Standard deviations.

particularly rapidly oxidized during extraction of uredospores. This point will be discussed later (p. 1334). Young spores contained more dehydroascorbate than older spores (Table II) but the difference was not significant in an unpaired '*t*' test ($P>0.05$). There was, however, a significant ($P=0.02$)

TABLE II

Dehydroascorbic acid in uredospores

Uredospores	Age	Extraction procedure	DHA, mg/100 g fresh wt.
Ungerminated	1 month	Ball mill	39.3
	1 year	Ball mill	28.7
	1 week	Blendor	47.5
	1 month	Blendor	31.5
	1 year	Blendor	30.1
	1 year	Blendor	19.0
			Mean 30.7
Germinated	1 month	Blendor	20.3
	1 month	Blendor	17.3
	1 year	Blendor	15.9
	1 year	Blendor	14.0
			Mean 16.8

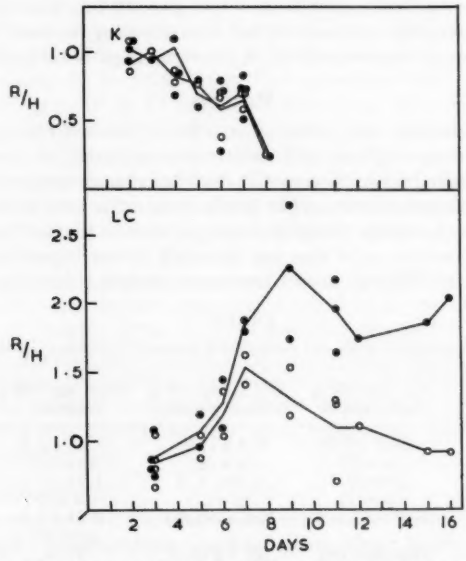

FIG. 1. Drifts in ascorbic acid in Khapli (top) and Little Club (bottom) after rust infection.

In this and subsequent figures, 'R/H' indicates the ratio of rusted to uninfected values, 'days' refers to days after inoculation, open circles refer to values calculated on a dry weight and solid circles to values calculated on a fresh weight basis.

decrease in dehydroascorbate during germination (Tables I and II). Oxidized glutathione was not found in measurable amounts in either infected or uninfected leaves, but was present in ungerminated uredospores (Table I).

The effects of rust infection on ascorbate, dehydroascorbate, and reduced glutathione are illustrated by the primary data for typical individual experiments given in Tables III and IV and by the curves for the relative concentrations (R/H) of these substances in rusted and uninfected plants in all experiments (Figs. 1, 2, 3). After infection ascorbate increased in Little Club, though the increase was later offset by the large increase in dry weight (Fig. 1 and Table III). On the other hand ascorbate decreased sharply in Khapli (Fig. 1 and Table III). Dehydroascorbate also increased after infection (Fig. 2 and Table III). After sporulation the DHA/AA ratio increased dramatically in both species of wheat, but the rise was very much delayed in Little Club

TABLE III

Ascorbic and dehydroascorbic acids in infected and uninfected wheat leaves

Variety	Days after inoculation	AA, mg/100 g fresh wt.		AA, mg/100 g dry wt.		DHA, mg/100 g fresh wt.		DHA, mg/100 g dry wt.	
		H	R	H	R	H	R	H	R
Little Club	3 (I)	37.3	32.7	318.8	259.5	9.0	8.5	76.9	67.4
	5 (Ps)	37.7	45.4	330.7	349.2	6.8	8.2	60.0	63.4
	7 (S)	47.4	86.0	412.1	589.0	9.8	15.2	85.2	104.1
	9 (S)	47.9	83.4	395.8	471.1	5.9	12.3	48.7	69.4
	11 (S)	45.9	75.9	428.9	308.5	8.8	15.7	82.2	63.8
Khapli	2 (I)	17.1	16.2	166.0	143.3	5.1	5.4	49.4	47.7
	4 (I)	38.9	33.4	344.2	269.3	4.2	5.4	37.1	43.5
	6 (Ps)	31.6	9.3	277.1	106.7	4.8	4.4	42.1	50.5
	7 (Ps)	23.5	12.4	215.5	129.0	2.7	4.5	24.7	47.3
	8 (Ps)	28.7	6.8	270.7	71.5	1.6	4.4	15.0	46.4

NOTE: In Tables III and IV, I = incubation stage, Ps = presporulation, S = sporulation.

TABLE IV

Glutathione in infected and uninfected wheat leaves

Variety	Days after inoculation	GSH, mg/100 g fresh wt.		GSH, mg/100 g dry wt.	
		H	R	H	R
Little Club	2 (I)	6.84	8.03	75.1	87.2
	6 (Ps)	5.84	6.96	62.8	55.6
	9 (S)	6.48	15.35	68.9	113.7
	10 (S)	7.80	20.10	76.4	157.0
Khapli I	3 (I)	8.50	9.00	64.4	72.0
	5 (Ps)	7.60	7.30	68.4	47.9
	6 (Ps)	8.10	6.20	71.6	56.2
	8 (S)	9.90	14.00	96.0	102.0
	9 (S)	8.00	18.20	72.0	134.0
Khapli	3 (I)	3.75	5.95	40.7	60.7
	6 (Ps)	4.54	4.48	52.2	46.6
	7 (Ps)	4.00	4.50	46.2	46.5

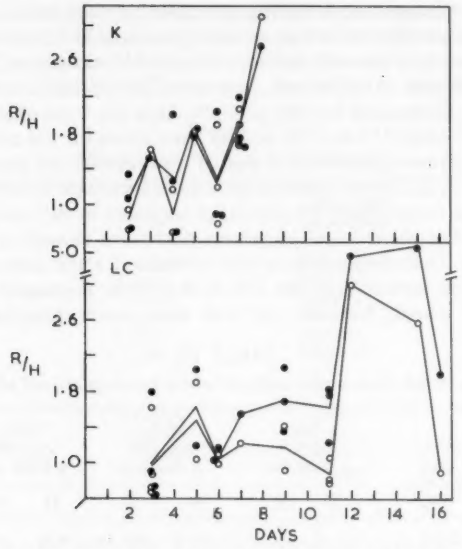

FIG. 2. Drifts in dehydroascorbic acid in Khapli (top) and Little Club (bottom) after rust infection.

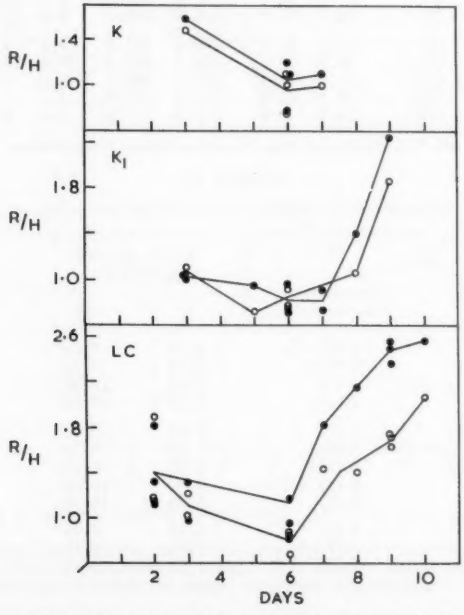

FIG. 3. Drifts in reduced glutathione in Khapli (top), Khapli I (center), and Little Club (bottom) after rust infection.

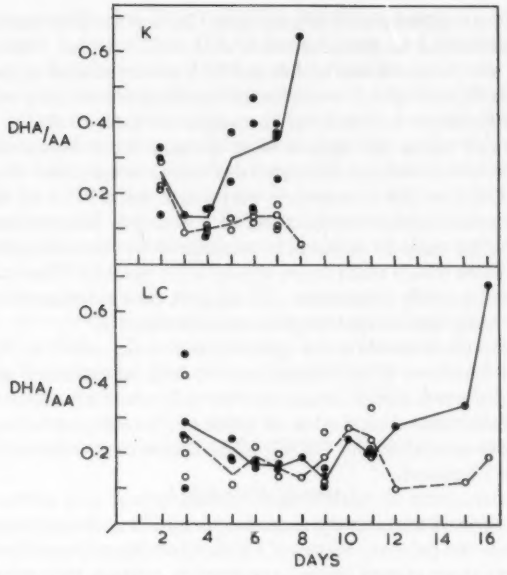

FIG. 4. Drifts in the ratio of dehydroascorbate: ascorbate in Khapli (top) and Little Club (bottom) after rust infection.

(Fig. 4). Analyses of Khapli leaves could not be continued after 8 days because of the death of the leaves.

Infection also increased the level of reduced glutathione in Little Club and Khapli I (Fig. 3 and Table IV). There was little or no effect in Khapli (Fig. 3), although it is possible that more-frequent analyses might reveal a statistically significant transient increase during the first few days after inoculation. Such an increase is suggested by the curves for Khapli and Little Club in Fig. 3. The increases in ascorbic acid in Little Club and of reduced glutathione in Khapli I and Little Club were associated with the development of uredospores 6 to 8 days after inoculation (Figs. 1 and 3). There was less dehydroascorbic acid in Khapli than in Little Club, but the difference did not reach a satisfactory level of significance. There was significantly less ($P=0.01$ in an unpaired 't' test) reduced glutathione in Khapli than in Little Club or Khapli I, but undue reliance should not be placed on this result because only five analyses were carried out with Khapli and these involved only two plantings made in the same month, whereas the greater number of analyses (10 to 14) carried out with the other two varieties were made over a much longer period (Table I).

Discussion

Barker and Mapson (1959) showed that a large fraction of the DHA extracted from plant tissue with acid at room temperature is formed during the extraction. They therefore suggested that DHA should be extracted at low

temperatures in an atmosphere of nitrogen. In the work reported here DHA and AA were estimated in extracts made with cold acid for 3 minutes only. It is possible that some conversion of AA to DHA occurred during this procedure, but extensive oxidation of AA would scarcely be expected in so short a period of time. It is difficult to account for the apparent lack of AA in uredospores. No AA was found when the spores were ground for 2 hours at 0° C under nitrogen. Either AA is oxidized in extracts of spores even under these conditions or the ratio of DHA to AA in spores is very high. Ascorbic acid reacts quickly with dichlorophenol–indophenol at a pH of 3.5 to 3.6. Measurement of reduction of the dye after only 15 seconds is considered to avoid errors due to other reducing substances which react more slowly with the dye. The values obtained for DHA were reasonably consistent and suggest that estimation of DHA after reduction with hydrogen sulphide gives reliable results.

Since the glyoxylase method for glutathione is regarded as highly specific (Patterson and Lazarow 1955) the apparent lack of oxidized glutathione in infected and uninfected leaves indicates that only small amounts were present, and also that there was no oxidation of reduced glutathione during the extraction. It is unfortunate that the GSSG/GSH ratios cannot be calculated from the information obtained.

From redox titrations of healthy and rusted wheat leaf extracts Kaul and Shaw (1960) suggested tentatively that the dominant poising system in extracts of mature, uninfected primary leaves of Little Club Wheat was the ascorbic acid system. Extracts from rusted leaves apparently contain two other systems in the reduced form. Figure 3 suggests that there may be a temporary rise in the concentration of GSH immediately after infection (2–3 days after inoculation), but the data are not extensive enough to allow a firm conclusion on this point. Figure 4 suggests a temporary rise in the DHA/AA ratio during this period. On the other hand, it is clear that when sporulation occurs at infections on Little Club there is a marked rise in GSH 6 to 7 days after inoculation and that the DHA/AA ratio does not rise much until 14 or more days after inoculation. In Khapli there is no rise in GSH and the DHA/AA ratio rises abruptly at 5 to 8 days after inoculation. There is a rough agreement between these results and those obtained by Kaul and Shaw (1960). In measurements obtained without the use of oxidation–reduction mediators (e.g. methylene blue) they found that there was an unreversed rise in E_h (pH 6.0) in leaf extracts of Khapli with the progress of infection. On the other hand, in extracts of Little Club the E_h first rose (4 days after inoculation) and then fell again to values near those for uninfected tissue. A final rise in E_h accompanied the death of the leaves. It is, of course, evident that an unreversed rise in oxidation–reduction potentials would be expected in dying tissue, no matter what the specific cause of death may be. The discrepancy between the apparent rise in GSH and that in the DHA/AA ratio noted above might disappear if the GSSG/GSH ratio were known. As the data stand, this is difficult to explain if the DHA/AA ratio is assumed to be related to the GSSG/GSH ratio through the activity of dehydroascorbic reductase (Mapson and Goddard 1951). Modern views on physiological relationships between auxin, ascorbate, and glutathione have been summarized by Galston and Purves (1960). Infection of

a susceptible host with rust first caused a possible decrease and subsequently a marked increase in free auxin (IAA) level (Shaw and Hawkins 1958; Sahai 1960). The auxin level of rusted tissue may thus be inversely related to the DHA/AA ratio, and this would be consistent with the view that auxin induces the inhibition of ascorbic acid oxidase (Marré and Arrigoni 1955). However, although evidence purporting to show that ascorbic acid oxidase functions as the terminal oxidase in rust-infected tissue (Kiraly and Farkas 1957) is not acceptable (Shaw 1959), the rate of oxidation of exogenously supplied ascorbate increases after rust infection and parallels rust development (Oaks 1958), and treatment with auxin increases ascorbic acid oxidase activity in tobacco tissue (Newcomb 1950).

Rust infection also lowers the C_6/C_1 ratio and thus probably involves an increase in glucose dissimilation via the pentose phosphate pathway (Shaw and Samborski 1957). This may result in an increase in the synthesis of ascorbate since 6-phosphogluconate has been found to be the ultimate precursor of ascorbate in strawberries (Loewus and Jang 1958). Ascorbic acid occurs in chloroplasts and its preservation may be a factor in the maintenance of the green islands that are so characteristic of rust infections on susceptible hosts.

Acknowledgment

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PHYSIOLOGICAL STUDIES ON A NECROTIC MUTANT OF CORN[1]

D. R. McCalla[2]

Abstract

The leaves of homozygous nec. 1 plants (a single-gene, necrotic maize mutant) develop visible necrotic symptoms when they are exposed to light for several days. Extensive analysis has failed to reveal any difference in chemical composition between leaves of normal plants and of homozygous nec. 1 plants before the necrotic symptoms are visible (prenecrotic leaves). Treatment of nec. 1 seedlings with various metabolites (B vitamins, purines, pyrimidines, amino acids, etc.) did not prevent the appearance of necrosis. The rate of photosynthesis of prenecrotic leaves is normal at low light intensities but only 20 to 50% of normal at saturating light intensity. $C^{14}O_2$ feeding experiments indicate that the photosynthetic carbon-fixing reactions function normally in the mutant. Hill reaction rates are also similar in mutant and normal plants, as is the metabolism of labelled inorganic phosphate. 3-(p-Chlorophenyl)-1,1-dimethylurea, which specifically inhibits photosynthesis to the extent of about 90%, delays the onset of visible necrotic damage and reduces the severity of subsequent necrotic symptoms. It is suggested that the basic lesion in nec. 1 plants lies in a reaction associated with photosynthesis and that it causes the accumulation of one or more toxic substances. These materials lower the rate of photosynthesis and damage cell membranes. The necrotic phenotype would appear to be the result of the breakdown of cell compartmentalization.

Introduction

Among the various lethal mutations which have been observed in higher plants are the so-called necrotics. The phenotype of these mutants is characterized by yellowing and browning of the leaf tissue which may be accompanied by liquid logging and exudate formation. In the field, necrotic seedlings may easily be missed altogether or dismissed as the result of cutworm or other damage. While various necrotic mutations are reported in the biological literature, it appears likely that many more such mutants have arisen but have gone unrecorded.

Wide crosses within the genus *Triticum* (wheat) have given rise to necrotic progenies (Morrison 1957). The phenotypes of the necrotic plants produced by such crosses vary from rapid, progressive, and lethal necrosis of the primary leaves, to late "firing" of the leaves at the flowering stage (Caldwell and Compton 1943). Hermsen (1960) has analyzed in considerable detail the genetics of semilethal necrosis in crosses of several varieties of *Triticum aestivum* (common wheat). Three complementary genes account for the results observed. The severity with which the necrotic symptoms appear is related to light intensity.

Tomato plants (*Lycopersicon esculentum*) which are homozygous for a recessive gene, ne, and which, in addition, carry the unlinked factor Cfp_1 (*Cladosporium* resistance introduced from *L. pimpinellifolium*) develop necrotic

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lesions on the leaves and pedicels (Langford 1948). The severity of these symptoms is increased by high temperature and light intensity.

Harrison (1960) has described the appearance of excrudescent necrotic lesions on the stems, leaves, and calyces of hybrid progeny from the cross *Antirrhinum orontium* × *A. meonanthum*. In this case the hybrid progeny are sterile so the genetic basis of the condition cannot be studied.

Chandler and Barton (1955) have reported exudate formation and necrosis in tetraploid *Plantago ovata* (plantain). Since the diploid plants from which the tetraploids were derived (by the use of colchicine) are perfectly normal, the factors leading to exudate formation and necrosis seem likely to be of a subtle nature. The authors studied the physiology of the condition in some detail and suggest that it may be associated with water relations.

E. G. Anderson (unpublished) has found a large series of necrotic mutants in maize. Some of these mutants are described in the Material and Methods section of this paper.

The physiology of necrotic mutants has been little studied and to the author's knowledge the nature of the basic lesions which cause this phenotype is completely unknown. The present study was aimed at determining the nature of the abnormality which leads to the necrotic phenotype in a maize mutant.

Materials and Methods

Plants

The necrotic mutant with which this study is mainly concerned arose in the maize (*Zea mays* L.) stocks of Dr. E. G. Anderson (unpublished). Genetic tests have shown that the necrotic condition appears in plants homozygous for a recessive gene designated by him necrotic_{rd}, but now to be known as necrotic 1 (nec. 1), which is located on the fourth chromosome. Highkin, Anderson, and McNutt (unpublished) carried out preliminary physiological studies on this mutant, which seems particularly suitable for such work since the first leaf appears normal for some time after it has unfolded. This makes it possible to work with tissue which is homozygous for the nec. gene before the many secondary changes inherent in the necrotic process are initiated. They found that etiolated plants do not become necrotic and that when heterozygous nec. 1 plants are crossed with plants heterozygous for an albino gene and the F_1 selfed, no necrotic plants are found among the albino progeny. Paper chromatographic studies of the exudate produced by nec. 1/nec. 1 plants showed the presence of glucose, a pentose, and many unidentified phenolic- and ninhydrin-positive compounds. Two other single-gene necrotic mutants were examined briefly during the present study. One, necrotic 6697, in which the condition appears before the first leaf has fully expanded, is located on chromosome 8. The other necrotic, 8376, is due to a recessive gene on chromosome 1 (Anderson, unpublished). The latter mutant has pale green leaves which become necrotic several days after emergence. In the following, the term necrotic, when used without further explanation, refers to nec. 1.

Seedlings were grown at 26° C in a controlled-temperature room in the Earhart Plant Research Laboratory (Went 1957). Artificial illumination provided light intensity of about 700 foot-candles and a daylength of 16 hours.

Seeds were planted in 4×8 in. plastic pans in a mixture of quartz and vermiculite and supplied twice daily with nutrient solution. In order to obtain known prenecrotic tissue, plants were generally left until the first visible sign of necrosis appeared; that is, until the tips of the first leaves of the necrotic segregants were visibly waterlogged. The plants were then harvested, the visibly damaged areas removed, and the remaining tissue used. Normal segregants were used for comparison. Where indicated, experiments were performed on leaf tissue taken from plants before any visible necrosis appeared. The rest of each plant was allowed to develop for several days and then examined for necrotic symptoms. In this way it was possible to relate the experimental results obtained to the phenotype of the leaf tissue used.

General

Leaf tissue was extracted with 80% ethanol and divided into acidic, basic, and neutral fractions by use of IR-45 and IR-120 ion exchange resins as described by Romberger and Norton (1961). Paper chromatographic techniques were used to separate and identify the constituents of each fraction.

Total soluble carbohydrate was determined by use of the anthrone reagent described by Morris (1948), and reducing sugar by the Somogyi (1945) procedure using the colorimetric reagent of Nelson (1944). Nitrogen was determined by Nesslerization of the ammonia formed by a micro-Kjehldahl digestion as outlined by Miller and Miller (1948). Total phenols were determined by the colorimetric procedure of Folin and Ciocalteu (1927).

Aliquots of extracts and aqueous solutions were plated on glass planchets at infinite thinness and counted to ±3% accuracy in a Nuclear Chicago D-47 gas flow counter fitted with a "Micromil" window. Carbon-14 in the insoluble residue was counted after combustion to $C^{14}O_2$ and precipitation as barium carbonate. The amount and distribution of carbon-14 on paper chromatograms was determined by means of a Nuclear-Chicago "Actigraph I" chromatogram scanner.

Determination of the Rate of Photosynthesis of Leaf Disks

Small pieces of leaf tissue (10 to 20 mg) were rapidly weighed and placed in Erlenmeyer flasks of appropriate volume (50 ml for 2 disks, 500 ml for 12), fitted with a stopper carrying a side tube with a small bulb. A small volume of solution containing carbon-14 labelled sodium bicarbonate was placed in the bulb, the tube sealed with a serum bottle cap, and about 0.1 ml of *N* sulphuric acid added by means of a hypodermic syringe. Ten milliliters of air was forced in to sweep the carbon dioxide into the flask. The flask was then illuminated from the bottom with light from a 150-watt reflector lamp 4½ inches from the flask and shielded with 2½ inches of water. A variac was used to control the intensity of illumination, which was measured with a Weston photometer calibrated in foot-candles. After 5 or 10 minutes of illumination the disks were removed from the flask, dropped into boiling 80% ethanol in a 15-ml graduated centrifuge tube, and exhaustively extracted. The volume of the extract was made up to 10 ml and aliquots were taken for the determination of carbon-14.

Determinations carried out simultaneously on paired pieces of tissue from

the same leaf agreed to within 10%. That the incorporation of carbon-14 from carbon dioxide into the 80% ethanol soluble material gives a meaningful comparison of normal and necrotic tissue is indicated by the fact that the fraction of the total carbon-14 incorporated into such material was found to be the same in the two tissues. The rate of dark fixation of carbon dioxide into ethanol-soluble compounds was never more than 1% of the rate in light of 1000 foot-candle intensity.

Chloroplasts were isolated in a sucrose medium at pH 7.8 (Jagendorf and Avron 1957) and the chlorophyll content of such preparations determined by optical density measurements at the isosbestic point of chlorophylls *a* and *b* (652 mμ) after disruption of the chloroplasts in 80% aqueous acetone (Arnon 1949). Hill reaction rates were determined by the spectrophotometric method of Krogmann and Jagendorf (1957) which employs ferricyanide as the oxidant.

Results and Discussion

Symptomatology

The first visible necrotic symptoms appear about 2 days after the first leaf has broken through the coleoptile. The tip of the first leaf becomes waterlogged and a characteristic red-brown exudate is produced in substantial quantities (0.05 ml from an affected area weighing 100 mg). The affected area enlarges progressively until the entire first leaf becomes necrotic. The second and third leaves are similarly afflicted. The fourth leaf rarely develops fully since the endosperm reserves are exhausted by this time. In the early stages of this process there is a very sharp line of demarcation between the waterlogged and the unaffected areas of the leaf. Over the course of about 2 days the waterlogged tissue turns yellow, then brown, and finally dries out.

For convenience, the successive stages of necrosis will be referred to as follows: (1) prenecrotic, tissue appears normal; (2) waterlogged, tissue waterlogged, exudate on leaf but still green; (3) yellowing; (4) brown and desiccated.

Normal and prenecrotic leaf tissue appear identical when examined under the microscope. Mesophyll cells of waterlogged tissue are somewhat shrunken and their contents appear very granular. No vacuole or nucleus can be seen in these cells after they are stained with neutral red. Sections cut from yellowed leaves show almost complete collapse of the mesophyll cells but the vascular bundles are relatively little affected except for the presence of abnormally granular cytoplasm in the bundle sheath. Thus it appears that necrosis in this mutant first involves the breakdown of mesophyll cells.

The residue after exhaustive 80% ethanol extraction of intact normal leaves is white while corresponding material from waterlogged necrotic leaves is a dirty gray color and that from some prenecrotic tissue is light gray. The black pigment is insoluble in all organic solvents tested and in aqueous alkali or acid. Visibly damaged coleoptile and root tissue have never been observed, even when the leaves of the same plant were completely necrotic.

Light Requirement

The fact that necrotic symptoms do not develop in etiolated plants was readily confirmed. Furthermore it was found that necrotic damage appeared

within 36 hours after 8-day-old, etiolated plants were placed in light of 700 foot-candles' intensity. The same result was obtained when excised etiolated leaves were maintained in light with their bases in water. If populations of light-grown plants were placed in the dark soon after expansion of the first leaf, all plants remained healthy. Necrotic mutants could be distinguished from normal plants in populations which were grown under red, green, or blue light so the quality of the light is not highly critical for the development of necrotic symptoms.

Illuminated prenecrotic areas of leaf tissue became necrotic in the light even after they had been plasmolyzed in 0.5 *M* mannitol.

Two further necrotic corn mutants were examined to determine whether light is necessary for the development of their necrotic phenotypes. Etiolated plants of both necrotic 8376 and necrotic 6697 appeared normal but developed necrotic leaf tissue shortly after being placed in the light.

It is well known that aminotriazole prevents the development of normal chloroplast pigments (Woodford *et al.* 1958). When corn seedlings are treated with aminotriazole at an early stage (10^{-3} *M* solution poured into the pan in which the seedlings are growing), the first leaf is entirely white. Necrosis has never been observed in such leaves. If, on the other hand, application of the aminotriazole is delayed until the first leaf has partially expanded it is possible to obtain plants in which the first leaf has a green tip and white base. Necrosis develops rapidly in the green areas of such plants but does not spread into the unpigmented portion. Thus it is clear that both light and chlorophyll are essential for the expression of the necrotic phenotype.

Chemical Changes

The most dramatic feature of the necrotic phenotype is the appearance of droplets of red-brown exudate on the surface of the leaves. The composition of this exudate was studied in some detail as was the composition of normal, prenecrotic, and waterlogged tissue. When four volumes of ethanol were added to a concentrated sample of exudate, a very much smaller precipitate formed than resulted when the same treatment was applied to an aqueous extract of normal leaf tissue (equal in area to the necrotic leaf from which the exudate was collected). This finding indicates that the cell membranes have become damaged so that they will allow water and other small molecules to pass freely but still prevent the passage of most high-molecular-weight materials. Hydrolysis of the precipitate from the exudate yielded only amino acids, indicating that a little protein is present but that polysaccharides of any considerable size are absent.

Paper chromatographic methods were used to study the low-molecular-weight compounds present in the exudate and tissue. Phenolic compounds with R_f values of 0.27 and 0.31 were identified as caffeic acid derivatives by comparison of their U.V. spectra with that of the pure acid and by hydrolysis to free caffeic acid which was then identified by chromatography in several solvents. The chromatographic properties and U.V. absorption spectrum of the R_f 0.81 phenol were found to be identical with those of an authentic sample of 1,3-dihydroxy-2-keto-6-methoxy-1,4-benzoxazine (DKB) which was kindly provided by R. H. Hamilton through R. S. Bandurski. The R_f 0.52

phenol gave the same spectrum as the benzoxazine derivative and probably represents its N-glucoside. Both of these compounds have been reported to occur in corn (Hamilton 1959). The R_f 0.85 phenol, which occurs only in the necrotic tissue, remains unidentified. It has an absorption spectrum characterized by a sharp peak at 283 mμ and gives a yellow color with diazotized sulphanilic acid. The sugars were identified by their paper chromatographic behavior in a number of solvents and by electrophoresis of their borate complexes.

Table I shows that changes in sugar and phenol composition take place as the tissue becomes necrotic. It will be noted, however, that normal and prenecrotic tissue cannot be distinguished on the basis of these compounds.

TABLE I

Composition of tissue, extracts, and autolyzate

Compound	R_f in BAW*	Relative amount in: Normal tissue	Prenecrotic tissue	Water-logged tissue	Exudate	Autolyzate
Phenols						
Caffeic acid derivative	0.27	++++	++++	++	++	+
Caffeic acid derivative	0.31	++++	++++	++	++	+
Glucoside of DKB†	0.52	++++	++++	+	Trace	Trace
DKB	0.81	—‡	—	+	+++	++++
Unknown phenol	0.85	—	—	+	+++	++++
Sugars						
Sucrose		+	+	—	—	Trace
Glucose		+++	+++	+	++++	++
Fructose		Trace	Trace	—	—	—
Ribose		—	—	+§	+	++
Other pentose		—	—	—	Trace	+
Uronic acid		—	—	—	Trace	+

*Butanol – acetic acid – water.
†1,3-Dihydroxy-2-keto-6-methoxy-1,4-benzoxazine.
‡Undetectable.
§Ribose present to the extent of 100 μg/g fresh wt.

Several ninhydrin-positive compounds, all but one of which could be matched with a known amino acid, were found on two-dimensional paper chromatograms. However, hydrolysis of the material from each individual spot in 6 *N* HCl at 105° C for 20 hours produced a mixture of amino acids. In each case the predominant amino acid found after hydrolysis appeared to be that inferred from the chromatographic behavior of the unhydrolyzed material. It seems probable that each zone consists of a free amino acid and one or more peptides with similar chromatographic properties. Similar peptides have been reported to occur in wheat leaves (Pharo and Claggett 1959). Since the ninhydrin-positive material of the exudate appeared to be very similar to that of normal tissue this aspect was not pursued further.

The red-brown "pigment" found in the exudate appears to be a complex mixture of polyphenolic materials. When it is chromatographed in butanol – acetic acid – water, it gives a phenol-positive streak extending from the origin to about R_f 0.10–0.15. Some of the colored material is able to pass

through a dialysis membrane, but a considerable portion of it is nondialyzable. The pigment is probably formed by the action of polyphenol oxidase on endogenous phenolic compounds. This enzyme has previously been reported to occur in corn leaves (Haskins 1955) and in the present study was found to be equally active in normal and prenecrotic tissue. It was also found that dialyzed extracts of normal corn convert caffeic acid to a colored, nondialyzable product. The benzoxazine derivative is also known to form colored polymers (R. S. Bandurski, private communication).

The data of Table I have already indicated that the chemical composition of prenecrotic leaves is similar to that of normal leaves. This conclusion is further supported by results of quantitative analysis which show that the two tissues cannot be distinguished on the basis of per cent dry weight, or content of soluble or insoluble nitrogen, of phosphorus, of reducing sugar, of total carbohydrate, or of total phenolic material. Yellowed tissue, however, was found to contain somewhat less of each of these substances.

Plastid pigments were extracted from normal and prenecrotic leaves and separated into carotene, xanthophyll, and chlorophyll fractions by chromatography on icing sugar columns (Tswett 1906). The absorption spectrum of each fraction from prenecrotic tissue was found to be identical with that of the corresponding normal fraction.

Toxicity of the Exudate

Highkin and McNutt found that exudate from waterlogged tissue caused small necrotic spots when applied to the leaves of unrelated normal seedlings. This observation was easily confirmed. However, in view of the known toxicity of many metabolites to plant tissue (Curtis 1943; Audus and Quastel 1947; and Schreiner and Reed 1908), it seems probable that the effect is nonspecific and unrelated to the basic cause of necrosis in the mutant. Further support for this view derives from the finding that the same type of necrotic leaf spot can be produced on normal leaves by an autolyzate of normal corn leaves (prepared by grinding surface-sterilized leaves in a mortar, allowing the paste to stand for 4 hours, straining the liquid through "Miracloth", heat-coagulating the protein, and centrifuging off the debris). Paper chromatographic studies (Table I, last column) of such an autolyzate indicate that the degradative changes involved in the formation of exudate in the necrotic mutant are similar to the autolytic processes in macerated leaves of normal corn.

Supplementation Experiments

Langridge (1958) has found mutants of the crucifer, *Arabidopsis thaliana*, which are unable to make one or another low-molecular-weight metabolite, for example thiamine, choline, cytidine, or biotin. The seeds which give rise to such plants apparently obtain sufficient of the substances from the maternal plant to allow germination and some seedling growth. Later growth, however, is very slow or stops altogether. In order to test the possibility that the lesion in necrotic plants is due to a lack of ability to make an essential metabolite, seeds were grown aseptically in 18×150 mm test tubes, containing 10 ml of mineral agar supplemented with 2% sucrose and other organic compounds as noted below. The tubes were covered with heavy aluminum foil and kept at a

temperature of 26° C and a light intensity of 700 foot-candles for a 16-hour day. Organic compounds added to the medium included B vitamins, amino acids, purines, pyrimidines, nucleosides, casein hydrolyzate, and yeast extract. None of these supplements prevented the development of necrotic seedlings so it appears that the lesion in the necrotic mutant is not due to the inability of the plant to make a common low-molecular-weight metabolite.

Respiration and Photosynthesis

The rate of respiration of prenecrotic leaf tissue is not significantly different from that of normal tissue. The respiratory quotients of the two tissues are also similar. Waterlogged tissue respires at about 30% of the normal rate while in yellowed tissue respiration has almost ceased (3% of normal). Sections cut from roots of normal and of necrotic plants respire at the same rate.

Preliminary measurements of carbon dioxide exchange rates in the light and dark, using a Beckman infrared carbon dioxide analyzer, indicated that the rate of photosynthesis of prenecrotic tissue in light of 1000 to 2000 foot-candles' intensity was only 20–50% of the rate observed when normal leaf tissue was used. This finding was confirmed in feeding experiments with carbon-14 labelled carbon dioxide. It was also found, however, that when light of lower intensity was used the rate of photosynthesis of the prenecrotic leaves was equal to that of normal leaves. Figure 1 shows the results of a typical experiment and indicates that the decrease in the rate of photosynthesis in the mutant at high light intensity is caused by a partial inhibition of the process in all chloroplasts and is not due to complete cessation of photosynthesis in a portion of the chloroplasts (or cells). If prenecrotic tissue did consist of such a mixture of normal and nonfunctional photosynthetic units, the rate

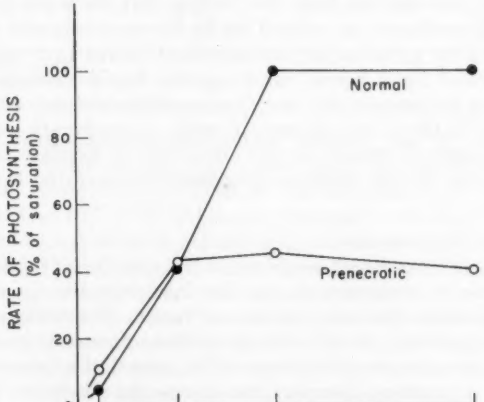

FIG. 1. Comparison of the rates of photosynthesis of leaf disks from normal and prenecrotic corn plants at various light intensities.

vs. intensity curve for prenecrotic tissue should be lower than the normal curve at all intensities and should level off at the same intensity as the normal curve. Other experiments have shown that the degree of inhibition of photosynthesis in prenecrotic tissue becomes progressively greater as the onset of visible necrotic symptoms approaches.

It is perhaps worthy of mention that only two classes of plants can be distinguished on the basis of rate of carbon dioxide fixation. The heterozygous, +/nec., plants do not differ from the normal homozygotes in this respect.

The distribution of carbon-14 in various fractions of the 80% ethanol soluble extract of prenecrotic leaf tissue after exposure to labelled carbon dioxide was found to be similar to that of normal tissue. Furthermore, the principal labelled compounds formed in each were sucrose, glucose, fructose, aspartic acid, alanine, glutamic acid, malic acid, succinic acid, and various unidentified sugar phosphates. The 80% ethanol insoluble fraction (mainly cell wall constituents, starch, and protein) contained the same proportion of the total carbon-14 in prenecrotic and normal leaves ($21 \pm 4\%$ and $24 \pm 3\%$ respectively). It must be concluded from these experiments that the photosynthetic carbon-fixing reactions all function normally in the mutant.

In order to gain further information concerning the integrity of biosynthetic processes in the necrotic mutant, the metabolism of glucose and of acetate was investigated.

Glucose (uniformly C-14 labelled) was fed to seedlings through the cut stem. After 2 hours in light of about 100 foot-candles' intensity, the tissue was extracted and the extract fractionated in the usual way. The results obtained with prenecrotic tissue were identical with those obtained with normal leaves. The principal labelled compounds were sucrose, fructose, raffinose, succinic, malic, and low R_f acids, aspartic acid, asparagine, glutamic acid, glutamine, alanine, tyrosine, phenylalanine, and tryptophan. The glucose and fructose moieties of sucrose were equally labelled.

Acetate-1-C-14 was fed in the same way, Again, no difference was found in the distribution of the carbon-14 in extracts of normal and prenecrotic tissue. In a separate experiment the lipids of tissue fed acetate-1-C-14 were extracted with chloroform: methanol (2:1, v/v) at 0° C with subsequent extractions at room temperature and 50° C. The combined extracts were evaporated under a stream of prepurified nitrogen. The residue was taken up in a few drops of benzene – isoamyl alcohol (1:1, v/v) and applied to silicic acid impregnated filter paper. The chromatograms were then developed in the diisobutylketone – acetic acid – water solvent (8:5:1, v/v) of Marinetti *et al.* (1957). Again the distribution of carbon-14 on the chromatograms from normal and prenecrotic tissue was identical. These results provide further evidence that the major carbon pathways operate normally in the mutant.

Effect of Inhibitors of Photosynthesis

Since both light and chlorophyll are required for expression of the necrotic phenotype, and since the photosynthetic apparatus becomes damaged before any other detectable changes occur, it is desirable to establish whether some lesion in the process of photosynthesis leads to the development of necrosis. The effect of inhibitors of photosynthesis on the development of the necrotic

phenotype was therefore examined.

3-(*p*-Chlorophenyl)-1,1-dimethylurea (CMU) is known to be a very selective inhibitor of photosynthesis which acts by blocking the electron transfer pathway in the chloroplast (Wessels and van der Veen 1956; Jagendorf and Margulies 1960). CMU treatment (4×10^{-4} *M* CMU solution poured into the pan in which the plants were growing just before the first leaf unfolded from the coleoptile) was found to inhibit 85 to 90% of photosynthetic carbon fixation but did not affect linear growth or dry weight accumulation of the leaves over a 5-day period. Increasing the CMU concentration 10-fold did not appreciably increase the extent of inhibition. CMU treatment delayed the appearance of necrotic symptoms by about 2 days and greatly reduced the rate at which necrosis later spread to the remaining leaf tissue. It seems reasonable to conclude that if photosynthesis could be completely inhibited by CMU no necrotic symptoms would appear. As previously noted by Minshall (1960) CMU-treated corn seedlings begin to degenerate after about 1 week and as a result the experiments could be continued for only a few days. Attempts to extend this type of study met with failure because the two other inhibitors tried, phenylurethane and iodoacetamide, were rapidly toxic even in concentrations at which photosynthesis was only slightly inhibited.

Hill Reaction and Photophosphorylation

From the data presented in previous sections, the process of photosynthesis appears to be involved in the development of necrosis in the mutant, but the pathways by which carbon compounds are formed appear to be normal. In an attempt to localize the lesion further, the Hill reaction and the process of photosynthetic phosphorylation were studied. Levine (1960) has recently found a mutant of the alga *Chlamydomonas* which is unable to carry out the latter reaction.

Table II gives data comparing the rates of reduction of ferricyanide by illuminated (at 1000 foot-candles) chloroplasts from normal and prenecrotic leaves. These data show that chloroplasts from prenecrotic leaves are able to carry out the Hill reaction and that, although the rate is less than normal in two of the four experiments, it is unlikely that the primary lesion in the mutant is due to the inability to carry out that part of photosynthetic electron transfer which makes up the Hill reaction.

Extensive attempts were made to compare the rates of photosynthetic phosphorylation by chloroplasts prepared from normal and prenecrotic

TABLE II

Reduction of ferricyanide (Hill reaction) by illuminated chloroplasts

Chloroplasts from	μM ferricyanide reduced per mg chlorophyll per hour			
	Expt. 1*	Expt. 2	Expt. 3	Expt. 4
Normal leaf	35	29	16	21
Prenecrotic leaf†	43	14	17	11
Waterlogged leaf	—	—	—	0.08

*Each experiment is the average of two determinations on the same chloroplast preparation. The individual experiments were done on different lots of seedlings.

†Rate of photosynthesis of leaves from which the prenecrotic chloroplasts were obtained was 20–40% of normal.

tissue, using the standard techniques developed for spinach (Arnon *et al.* 1954; Jagendorf and Avron 1957) and applied successfully to a number of other plants with soft leaves (Whatley *et al.* 1960). However, no esterification of inorganic phosphate could be detected even in the case of preparations from normal corn. (Rates similar to those reported in the literature were obtained with spinach chloroplasts.) So far as the author is aware, there are no published reports of photophosphorylation by chloroplasts from a monocotyledon. It seems that special techniques will be necessary with such material.

Because it proved impossible to study photosynthetic phosphorylation in the mutant by use of a cell-free system, experiments comparing the metabolism of inorganic phosphate containing P-32 by normal and prenecrotic leaves were carried out. The labelled phosphate was fed to intact plants through their roots and to excised leaves through their bases. With either of these procedures it was necessary to allow considerable time for uptake of the tracer. Short-term (10-minute) experiments were therefore carried out by floating leaf disks on a solution of radioactive phosphate which contained 0.05% "Tween 80" to reduce the surface tension. After the period allowed for feeding and metabolism, the tissue was killed and extracted in the usual way. In early experiments, the extracts were chromatographed in the solvent used by Loughman and Martin (1957) to separate phosphate-containing compounds in barley (*t*-butanol – water – picric acid 80:20:2, v/v/w). This solvent gave fairly good separation of the material into nucleotide, sugar phosphate, and inorganic phosphate zones, but ran slowly. In addition the presence of picric acid on the chromatogram interfered with the use of spray reagents. For these reasons the *n*-butanol – acetic acid – water (74:19:50, v/v) solvent devised by Schwink (1956) was used. The ATP[3] zone was eluted from the paper and electrophoresed in pH 4.6 buffer in order to confirm the identity of ATP. The results of these experiments show no consistent differences in the proportion of P-32 incorporated into ATP, other nucleotide phosphates, or sugar phosphates, between prenecrotic and normal leaf tissue in either the light or dark.

Conclusions

The intimate relation of the primary lesion to photosynthesis in the nec. 1 mutant is suggested by the complete absence of necrotic symptoms from illuminated, chlorophyll-free leaf tissue and from etiolated leaves. Mitigation of the necrotic phenotype following inhibition of 85 to 90% of normal photosynthetic carbon fixation with CMU provides further convincing evidence for this view.

A good hypothesis as to the nature of the primary biochemical lesion seems to be the following: the lesion causes some compound which is ordinarily present in very small amounts to accumulate to toxic concentrations during photosynthesis and gradually damage the photosynthetic apparatus. This compound could be a minor carbon-containing product of photosynthesis or a compound which accumulates because of some defect in a reaction involv-

[3] Adenosinetriphosphate.

ing either the reducing or oxidizing product of water photolysis. That it cannot be one of the major products of photosynthetic carbon fixation is indicated by the results of $C^{14}O_2$ feeding experiments. This same toxic compound must cause, directly or indirectly, the breakdown of the membranes of the mesophyll cells. The presence of an abnormal dark pigment on the residue of prenecrotic leaves before any other visible damage has occurred suggests that the tonoplast becomes leaky and allows mixing of cytoplasmic and vacuolar material before the plasma membrane breaks down.

With the breakdown of cell compartmentalization, mixing of normally separated cellular components takes place. It seems probable that here, as with other damaged tissues, normally masked degradative enzymes become activated. It is little wonder then that extensive chemical changes are taking place by the time the tissue becomes visibly necrotic.

In some of the other necrotic corn mutants and in some of the pathogen-induced necroses no exudate is produced and the tissue has a dry appearance. The extensive exudate production by homozygous nec. 1 plants probably results from continued water movement due to root pressure through the relatively undamaged xylem elements at the time the mesophyll cells have become leaky. Eventually, of course, the xylem, too, is damaged and the tissue becomes dried out.

The observation of two other mutants in which necrosis develops only when plants are illuminated suggests that there may be a whole class of mutants in which a necrotic phenotype is caused by defects in the photosynthetic system.

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THE PHYSIOLOGY OF HOST–PARASITE RELATIONS

VII. THE EFFECT OF STEM RUST ON THE NITROGEN AND AMINO ACIDS IN WHEAT LEAVES[1]

MICHAEL SHAW AND NICHOLAS COLOTELO[2]

Abstract

The dry weight of leaf disks (2.8 mm in diameter) bearing pustules of stem rust and cut from primary leaves of Little Club wheat increased up to 2.5-fold, but the respective weights of host and parasite cannot be measured. By removal of the ectoparasitic mycelium of *Erysiphe* it was shown that approximately half of the increase in weight at infections of this organism on barley leaves was contributed by the fungus and half by the host.

At infections of stem rust on Little Club there was a striking increase in total N per gram fresh weight and an increase in the ratio of soluble to insoluble N. Quantitative paper chromatographic analyses revealed a fourfold increase in free amino acids and nearly a twofold increase in protein amino acids per gram fresh weight by 9 days after inoculation. The most striking increases occurred in free glutamine, γ-aminobutyric acid, threonine, and those amino acids present only in trace amounts before inoculation, particularly the basic and aromatic acids. Only slight and transitory increases in N occurred at infections on Khapli and the infected leaves quickly reached a stage at which soluble and insoluble N and the ratios of soluble N to insoluble N and free to protein amino acids declined drastically. In Khapli, well-defined increases occurred in free glutamine and γ-aminobutyric acid. Particularly striking decreases occurred in glutamate, serine, threonine, glycine, and the leucines. The results are discussed.

Introduction

It has been known for a long time that the total N content and ratio of soluble to insoluble N are increased in the later stages of development of rust on susceptible varieties of cereals (Gassner and Franke 1938).

The analyses reported here were undertaken to obtain quantitative information about the concentration and composition of the 'free' and 'combined' or 'protein' amino acids in rust-infected leaves of Little Club (susceptible) and Khapli (resistant) wheats. The experimental work was completed in 1956 (Colotelo 1956). The results of a similar but qualitative study of eight other varieties of wheat have been published by Rohringer (1957).

Materials and Methods

Plant Material

Greenhouse-grown plants of Khapli (rust reaction type 1) and Little Club (rust reaction type 4) wheats were infected with Race 15B of *Puccinia graminis tritici*. Atlas barley was infected with *Erysiphe graminis hordei*. The methods of treating the seed and growing and inoculating the plants, as well as the type of infections which developed, have already been described (Shaw and Samborski 1956), the only innovation being the use of supplementary lighting

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from October until April. This was supplied for 12 hours daily at an intensity of approximately 1000 ft-c from 'daylight' fluorescent lamps. Sporulation began 6–7 days after inoculation of Little Club. Little or no sporulation occurred on Khapli.

Harvesting

Samples of uninfected and heavily infected first-seedling leaves were harvested at 1- to 3-day intervals after inoculation. Heavily infected leaves of Khapli died 7 to 10 days after inoculation. Infected leaves of Little Club remained alive for 15 to 20 days or longer (see Table II in Part II). The uninfected basal sections of infected leaves were discarded and loose spores and any adhering flakes of gelatin (which was used as a 'sticker' in the procedure for inoculation) were wiped off the leaves. These were then cut into small pieces. This material is referred to in the text as the 'bulk' samples. Subsamples were taken for determination of fresh and dry weights (Samborski and Shaw 1956) and total nitrogen (see below). The remaining material was sealed in weighing bottles and stored in Dry Ice until the analyses could be carried out. Fresh and dry weights and total nitrogen contents were also determined for samples of leaf disks (diam. 2.8 mm or 5 mm) cut from healthy and uninfected leaves as described earlier (Samborski and Shaw 1956). This is referred to in the text as the 'leaf-disk' technique.

Extraction and Analysis

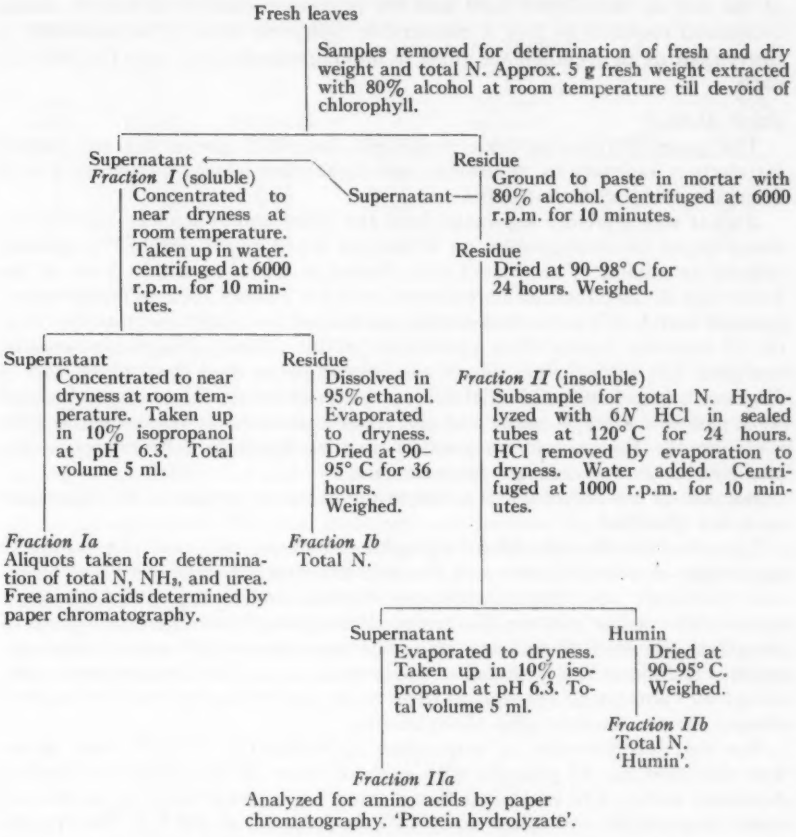
The leaf material was treated so as to obtain a fraction (I) soluble in 80% alcohol and an alcohol-insoluble fraction (II). Fraction I was split into water-soluble (I*a*) and water-insoluble (I*b*) parts. Fraction I*a* was analyzed for free amino acids by paper chromatography, and also for urea and ammonia. Fraction II was hydrolyzed with 6*N* HCl in sealed tubes at 120° C for 24 hours. The acid was removed by evaporation. The hydrolyzate (fraction II*a*) was analyzed for amino acids by paper chromatography and is referred to in the text as the 'protein' hydrolyzate. The dry weights of fractions I*b*, II, and II*b* (humin), were determined so that the analytical results could be expressed per unit weight of the fraction or of the original tissue. The total nitrogen contents of fractions I*a*, I*b*, II, and II*b* were also determined by a modification of the semimicro-Kjeldahl method given in A.O.A.C. (1945). Mercuric oxide was used as the catalyst. The entire procedure for extraction and analysis is summarized in the accompanying flow sheet.

Paper Chromatography of Amino Acids

The amino acids in fractions I*a* and II*a* were estimated and separated by quantitative two-dimensional partition chromatography on washed and buffered Whatman No. 1 filter paper sheets (Thompson and Steward 1951) using phenol–water (4:1) as the first and collidine–lutidine–water (1:1:2) as the second irrigating solvent. The solvents were purified by distillation before use. The dried papers were sprayed with 2% ninhydrin in ethanol and developed in an atmosphere of carbon dioxide and ethanol at 60° C (Thompson and Steward 1951).

The ninhydrin-positive spots were then cut out and the color eluted from them with 50% ethanol. The optical densities of these and eluates of appro-

FLOW SHEET FOR ANALYSIS OF WHEAT LEAVES


Fresh leaves
Samples removed for determination of fresh and dry weight and total N. Approx. 5 g fresh weight extracted with 80% alcohol at room temperature till devoid of chlorophyll.
Supernatant
Fraction I (soluble)
Concentrated to near dryness at room temperature. Taken up in water. centrifuged at 6000 r.p.m. for 10 minutes.
Supernatant
Residue
Ground to paste in mortar with 80% alcohol. Centrifuged at 6000 r.p.m. for 10 minutes.
Residue
Dried at 90–98° C for 24 hours. Weighed.
Supernatant
Concentrated to near dryness at room temperature. Taken up in 10% isopropanol at pH 6.3. Total volume 5 ml.
Residue
Dissolved in 95% ethanol. Evaporated to dryness. Dried at 90–95° C for 36 hours. Weighed.
Fraction II (insoluble)
Subsample for total N. Hydrolyzed with 6N HCl in sealed tubes at 120° C for 24 hours. HCl removed by evaporation to dryness. Water added. Centrifuged at 1000 r.p.m. for 10 minutes.
Fraction Ia
Aliquots taken for determination of total N, NH3, and urea. Free amino acids determined by paper chromatography.
Fraction Ib
Total N.
Supernatant
Evaporated to dryness. Taken up in 10% isopropanol at pH 6.3. Total volume 5 ml.
Humin
Dried at 90–95° C. Weighed.
Fraction IIb
Total N.
'Humin'.
Fraction IIa
Analyzed for amino acids by paper chromatography. 'Protein hydrolyzate'.

priate 'blank' portions of the chromatograms were measured with a Beckman Spectrophotometer (Model DU) at 5700 Å. The amount of each amino acid was then determined by reference to standard curves. These were prepared by chromatographing known amounts of mixtures of authentic compounds. Values from three separate chromatograms were used for the determination of each point on a standard curve. Aliquots equivalent to 3–10 mg dry weight of the original leaf tissue were employed in chromatographing the free amino acids. The use of larger aliquots resulted in excessive 'streaking'. Smaller aliquots (0.4–0.85 mg) were employed for the protein hydrolyzate. Amino acids present on the chromatograms in amounts too small to allow accurate measurement are listed as present in 'trace' amounts in the appropriate tables. A quantitative estimate of the maximum possible concentration of a

'trace' of any individual amino acid is readily arrived at from a knowledge of the size of the aliquot used and the minimum amount of known amino compound required to give a measurable ninhydrin spot. This procedure is convenient as an approximate guide in making calculations from the primary data.

Other Methods

The paper chromatographic technique described above did not permit satisfactory estimations of proline and asparagine which, on paper, give a brownish color with ninhydrin.

Proline was therefore separated from the other amino acids by one-dimensional paper chromatography on Whatman No. 1 paper with 77% aqueous ethanol as solvent. The ethanol was allowed to travel to within 1 cm of the lower edge of the paper, which was then dried for 2 hours at room temperature, sprayed with 0.2% isatin in acetone, and heated in a steam bath at 74–76° C for 20 minutes. Under these conditions proline forms a bright blue-colored complex. The optical densities of the colored areas were determined with a Photovolt densitometer (Model 525) at 1-mm intervals. The values obtained were plotted on graph paper and the areas under the curves measured with a planimeter. Proline concentrations were then determined by reference to a standard curve for area vs. concentration.

Because of an unfortunate accident quantitative estimates of asparagine were not obtained.

The two-dimensional chromatographic technique did not give complete separation of phenylalanine and the two leucines. *Phenylalanine* was therefore selectively adsorbed on activated charcoal and eluted with a phenol – acetic acid – water mixture (Partridge 1949). Quantitative determinations of phenylalanine were then made using the two-dimensional paper chromatographic technique and a standard calibration curve. The *leucines* were estimated by subtracting the optical density for phenylalanine from the optical density for the leucines plus phenylalanine.

For the determination of *tryptophan* approximately 5 g of fresh leaves was extracted for 15 minutes with each of three 50-ml aliquots of boiling deionized water. The combined water extract was evaporated to dryness at room temperature and taken up in 10% isopropanol at pH 6.5. The tryptophan was adsorbed on charcoal, eluted (Partridge 1949), and estimated by the paper chromatographic technique.

Ammonia and *urea* were estimated by the Conway diffusion method (Conway 1947). Urea nitrogen was converted to ammonia with urease. A standard curve was prepared using solutions of ammonium sulphate.

Results

Changes in Dry Weight, Total N, and the Ratio of Soluble to Insoluble N

It is impossible to separate the mycelium of *Puccinia* quantitatively from the tissue of the wheat leaf and it is therefore impossible to measure the separate contributions of host and parasite to the increases in dry weight or N content that follow infection with rust. On the other hand, the ectoparasitic mycelium of *Erysiphe* can be readily removed from the surface of parasitized

TABLE I

The effect of mildew infection on the dry weight of the first seedling leaf of Atlas barley

	Dry weights (mg/0.185 cm²)					
Days after inoc.	1 Healthy (H)	2 Mildewed (M)	3 M−H	4 Mildew removed (MR)	5 M−MR	6 M−MR, as % of M−H
4	4.47	4.35	−0.12	4.31	0.04	—
7	4.57	5.75	1.18	5.11	0.64	54.2
9	4.39	5.97	1.58	5.04	0.93	58.9
11	4.26	6.17	1.91	5.47	0.70	45.8
13	4.70	7.35	2.65	5.99	1.36	51.3
					Mean	52.6

leaves by rubbing them gently between the forefinger and thumb and brushing with a camel's hair brush.

The effect of a very severe infection with *Erysiphe* on the dry weight of barley leaves is shown by the data in Table I. Column 1 gives the mean values for 'rubbed' and untreated, uninfected leaves. A paired '*t*' test of the data showed that rubbing had no effect on the dry weight of uninfected leaves ($P > 0.05$). Column 6 shows that approximately 53% of the total increase in dry weight caused by infection is to be attributed to the weight of the parasite itself. This is an underestimate, because the 'rubbing' technique does not remove haustoria from within the epidermal cells of the host. The error thus introduced is clearly not large, as may be judged from microscopic examination of epidermis stripped from infected tissue.

Using the 'leaf-disk' technique large increases in dry weight and total N per unit were readily demonstrated at rust infections on Little Club (Fig. 1). At infections on Khapli there were rapid and early losses in both dry weight

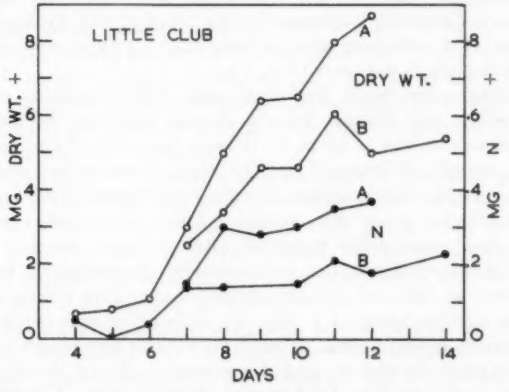


FIG. 1. Changes (rusted minus uninfected) in dry weight and total N content of leaf disks (diameter, 2.8 mm) after rust infection of Little Club wheat. In this and subsequent figures, 'days' refers to days after inoculation.

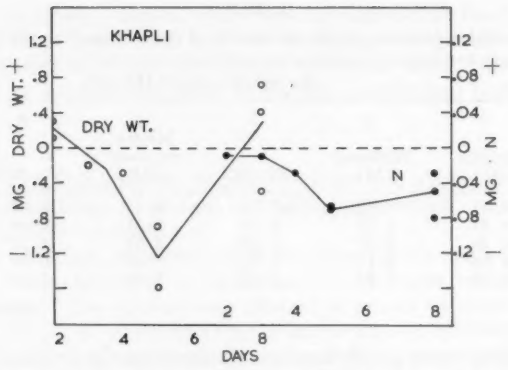


FIG. 2. Changes (rusted minus uninfected) in dry weight and total N content of wheat leaf disks (diameter, 2.8 mm) after rust infection of Khapli wheat.

and total N, from which there was usually no recovery (Fig. 2). Increases in the fresh weight of Little Club leaves did not exceed 35% in these experiments. The fresh weight of infected leaves of Khapli sometimes increased and sometimes decreased, but did not alter by more than 10% of the initial weight.

Two series of observations obtained by the 'leaf-disk' technique are recorded here for Little Club (Fig. 1). In series A the nitrogen content of the control plants had a mean value of 0.322 and ranged from 0.30 to 0.38 mg per 1.85 cm^2 of leaf surface. The dry weights ranged from 4.3 to 5.2 mg with a mean value of 4.69 mg. In series B the nitrogen content of the control plants averaged only 0.223, ranging from 0.21 to 0.24 mg per 1.85 cm^2. Similarly the dry weight averaged only 4.38 mg and ranged from 3.6 to 4.9 mg per 1.85 cm^2. It will be noted that the increases in dry weight and nitrogen content per unit area after rust infection were substantially greater and continued for a longer period of time in series A (Fig. 1).

Measurements using both 'leaf-disk' and 'bulk' samples of healthy and rusted Little Club and Khapli wheats showed that the N content per gram dry weight fell as the leaves aged. In Khapli the ratio of the nitrogen content per gram dry weight of infected and uninfected leaves fell steadily to about 0.80 (Fig. 3). In the later stages of rust development on Little Club, the nitrogen content per gram dry weight tended to remain constant or even rose slightly and eventually exceeded the nitrogen content of uninfected leaves, which declined slowly but continuously. Accordingly, the ratio of the nitrogen content of infected and uninfected leaves first fell to about 0.90 and then increased again to about 1.1 (Fig. 3). This point was reached at different times in different experiments, depending on the environmental conditions, nutritional status of the plants, and the intensity of rust development. It was not reached in series A (Fig. 1), but was almost attained in series B (Fig. 1) (N content per g dry weight in rusted tissue = 97% of N in uninfected tissue at 14 days after inoculation).

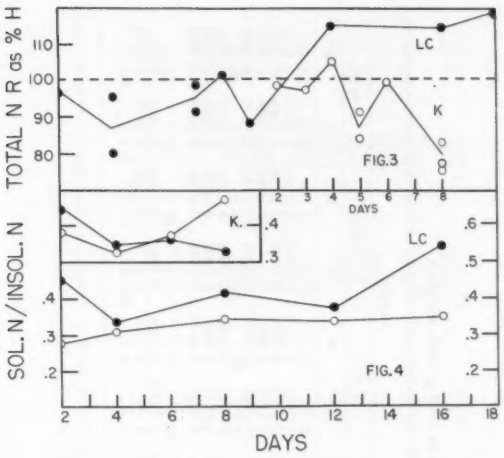


FIG. 3. Drifts in total N in Little Club and Khapli wheat after rust infection.
FIG. 4. Drifts in the ratio of soluble N to insoluble N in Little Club and Khapli (insert) wheat.

In Little Club the ratio of soluble to insoluble N (Fig. 3) was higher in infected than in healthy plants throughout the course of rust development. In Khapli the ratio was higher in infected tissue at 2 days after inoculation, but subsequently fell to about two-thirds of the value for healthy tissue (Fig. 3).

Summary of Nitrogen Fractions in Healthy and Rust-Infected Leaves

Rust-infected and uninfected leaves of Little Club and Khapli were analyzed for total, soluble, and insoluble N as well as for urea, ammonia, and free and combined amino acids at intervals between 2 and 9 days after inoculation. Table II constitutes a balance sheet for the nitrogen fractions measured in these experiments. This provides a background of information against which the detailed analyses of free and combined amino acids presented in the next section may be evaluated.

The recovery of total N as soluble and insoluble N (see flow sheet) averaged only 88.7% for Little Club and 89.5% for Khapli and was in some instances as low as 83%. Thus losses averaging 10–11% presumably occurred during the handling procedures, perhaps particularly with the smaller soluble fractions. A small proportion of the total may have been lost as ammonia, but there is no evidence to support this suggestion.

The free amino acids together with urea and ammonia accounted for only 15–40% of the total soluble N. The figures for free amino acids were obtained from the paper chromatographic estimates of individual amino acids (see below) and do not include those acids present only in 'trace' amounts in infected and uninfected plants or the tryptophan measured in rusted Little Club at 7 and 9 days after inoculation (see Table III).

TABLE II

Nitrogen fractions in uninfected and infected wheat leaves (g/100 g fresh weight)

	Little Club						Khapli							
	2 days*		7 days		9 days		2 days		3 days		5 days		8 days	
	H	R	H	R	H	R	H	R	H	R	H	R	H	R
Dry weight	8.88	9.22	9.48	12.58	9.45	21.11	9.89	10.22	9.25	9.78	10.65	11.12	10.59	10.59
Total N	0.6121	0.6205	0.5573	0.6766	0.5507	1.0938	0.6280	0.6382	0.5044	0.5195	0.5336	0.5109	0.5674	0.4723
Water-sol. N	0.0973	0.1671	0.1139	0.1514	0.1235	0.2629	0.1513	0.1634	0.0973	0.1018	0.0923	0.0828	0.1560	0.0959
95% alcohol sol. N	0.0166	0.0178	0.0153	0.0188	0.0217	0.0201	0.0088	0.0096	0.0091	0.0042	—	0.0066	0.0136	0.0057
80% alcohol insol. N	0.4160	0.4121	0.3931	0.4356	0.3165	0.6200	0.4233	0.3944	0.3475	0.3621	0.3768	0.3540	0.3575	0.3070
% Recovery total N	86.6	96.2	93.7	89.5	83.8	82.6	92.9	88.9	90.0	90.1	87.9	86.8	92.9	86.5
Soluble amino acid N†	0.0190	0.0293	0.0162	0.0406	0.0143	0.0733	0.0151	0.0102	0.0197	0.0182	0.0155	0.0163	0.0182	0.0156
Urea N	0.0018	0.0011	0.0017	0.0017	0.0006	0.0022	0.0019	0.0019	0.0012	0.0016	0.0005	0.0020	0.0023	0.0033
Ammonia N	0.0043	0.0090	0.0050	0.0041	0.0061	0.0055	0.0063	0.0102	0.0126	0.0135	0.0128	0.0133	0.0079	0.0066
% Recovery water-sol. N	25.8	23.6	20.1	30.6	17.0	30.8	15.4	13.6	34.4	32.7	31.2	38.2	18.2	26.6
Insol. amino acid N	0.3415	0.3823	0.2898	0.3741	0.3293	0.5239	0.3843	0.3529	0.2340‡	0.3309	0.3965	0.3203	0.3802	0.2891
Humin N	0.0143	0.0157	0.0171	0.0174	0.0118	0.0182	0.0239	0.0230	0.0187	0.0171	0.0171	0.0240	0.0210	0.0213
% Recovery insol. N	85.5	96.6	78.1	89.9	107.8	87.4	96.4	95.3	72.7‡	96.1	109.8	97.3	112.2	101.1

*Days after inoculation.
†From paper chromatographic determinations of constituent amino acids.
‡Excludes leucines and phenylalanine, which were accidentally lost.

TABLE III

Free amino acids (g/100 g fresh weight)

	Little Club								
	2 days			7 days			9 days		
	H	R	R/H	H	R	R/H	H	R	R/H
Dry weight	8.88	9.22	1.04	9.48	12.58	1.33	9.45	21.11	2.23
Aspartic	.0147	.0164	1.12	.0171	.0384	2.25	.0157	.0716	4.56
Asparagine	++	++	—	++	++	–	++	++	
Glutamic	.0438	.0584	1.33	.0577	.0638	1.11	.0521	.0969	1.86
Glutamine	.0056	.0107	1.91	.0132	.0457	3.46	.0103	.1170	11.36
Lysine	.0079	.0138	1.75	Tr.	.0152	–	Tr.	.0230	–
Arginine	.0054	.0277	5.13	Tr.	.0146	–	Tr.	.0283	–
Proline	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–
Phenylalanine	.0077	.0106	1.38	–	.0102	–	–	.0137	–
Tyrosine	Tr.	.0054	–	Tr.	Tr.	–	Tr.	.0099	–
Tryptophan	–	–	–	Tr.	.0224	–	Tr.	.0224	–
Serine	.0365	.0326	0.89	.0202	.0401	1.99	.0195	.0540	2.77
Threonine	.0040	.0078	1.95	.0013	.0065	5.00	.0010	.0108	10.80
Alanine	.0099	.0137	1.38	.0208	.0254	1.22	.0167	.0277	1.66
Glycine	Tr.	.0014	–	.0006	.0010	1.67	.0007	.0034	4.86
Leucines	.0062	.0113	1.82	Tr.	.0068	–	Tr.	.0093	–
γ-Aminobutyric	.0012	.0026	2.17	.0013	.0069	5.31	.0010	.0156	15.6
Valine	.0050	.0112	2.24	Tr.	.0043	–	Tr.	.0072	–
Total	.1479 (1.665)	.2236 (2.425)	1.51 (1.46)	.1322 (1.395)	.3013 (2.395)	2.28 (1.72)	.1170 (1.238)	.5108 (2.420)	4.37 (1.95)

TABLE III (*Concluded*)

Free amino acids (g/100 g fresh weight)

	Khapli											
	2 days			3 days			5 days			8 days		
	H	R	R/H	H	R	R/H	H	R	R/H	H	R	R/H
Dry weight	9.89	10.22	1.03	9.25	9.78	1.06	10.65	11.12	1.04	10.59	10.59	1.00
Aspartic	.0210	.0164	0.78	.0172	.0170	0.99	.0150	.0112	0.78	.0178	.0159	0.89
Asparagine	++	++	–	++	++	–	++	++		++	++	–
Glutamic	.0582	.0533	0.92	.0553	.0484	0.88	.0484	.0326	0.67	.0535	.0316	0.59
Glutamine	.0008	Tr.	–	.0126	.0126	1.00	.0113	.0172	1.52	.0111	.0195	1.76
Lysine	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–
Arginine	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–
Proline	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–
Phenylalanine	–	–		–	–	–	–	–	–	Tr.	.0040	–
Tyrosine	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–
Tryptophan	N.A.	N.A.	–	N.A.	N.A.	–	–	.0003	–	–	.0004	–
Serine	.0182	.0155	0.85	.0031	.0198	6.39	.0161	.0172	1.07	.0293	.0084	0.29
Threonine	Tr.	Tr.	–	.0027	.0025	0.93	.0027	.0009	0.33	.0015	.0020	0.13
Alanine	.0212	.0183	0.86	.0252	.0275	1.09	.0229	.0275	1.20	.0183	.0189	1.03
Glycine	.0050	Tr.	–	.0053	.0053	1.00	.0014	.0012	0.86	.0024	.0004	0.17
Leucines	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	.0019	.0050	2.63
γ-Aminobutyric	Tr.	Tr.	–	.0027	.0043	1.59	.0030	.0085	2.83	.0052	.0089	1.71
Valine	Tr.	Tr.	–	.0017	.0006	0.35	.0005	.0012	2.40	.0019	.0026	1.37
Total	.1244 (1.258)	.1035 (1.013)	0.83 (0.81)	.1258 (1.360)	.1380 (1.411)	1.10 (1.04)	.1213 (1.139)	.1178 (1.059)	0.97 (0.93)	.1429 (1.349)	.1176 (1.110)	0.82 (0.82)

NOTE: Figures in brackets are g/100 g dry weight.
Tr. = trace.

The results suggest an increase in urea with the progress of infection, particularly in the resistant variety, Khapli. Ammonia was not increased by rust infection, but uninfected leaves of Khapli averaged double (0.099 g NH_3 N per 100 g fresh weight) the ammonia content of Little Club.

The recovery of 80% alcohol insoluble N as combined amino acids and humin averaged 91% in Little Club and 101% in Khapli, excluding one instance with Khapli for which measurements of the leucines and phenylalanine were not obtained. It will be noted that individual recoveries of insoluble N ranged as high as 112%. This reflects the inaccuracies of the estimates of individual amino acids by the paper chromatographic technique. Such estimates are probably in error by $\pm 10\%$ to $\pm 15\%$.

The drifts in total, soluble, and insoluble N already described (Figs. 1 to 4) are again illustrated by the data in Table II. At the same time infection also caused extensive increases in free and combined amino acid N and in the ratio of free to combined amino N in Little Club but not in Khapli.

Amino Acid Analyses

Analyses were carried out on three occasions with Little Club and four with Khapli. The data for Khapli represent two plantings, made in March and June 1955, but are considered sufficiently alike to be grouped as a single experiment. The data for Little Club are from a single planting made in April. All primary data are presented in Tables III and IV. They allow a comparison of the actual and relative compositions of the free and combined amino acids in uninfected leaves of the two species of wheat and an examination of the effect of rust infection on the two amino acid fractions.

It is convenient first to consider the analytical results for uninfected Little Club and Khapli. The mean concentrations of individual amino acids are given in Table V. In calculating these from the primary data an appropriate value has been assigned to each amino acid listed as present in 'trace' amounts in Tables III and IV (see *Methods*). Methionine and histidine, which develop only faintly colored spots with ninhydrin on chromatograms developed in collidine–lutidine, were not detected in any of the extracts. However, no special attempt was made to demonstrate the presence of these amino acids by chromatography of larger aliquots of the extracts. Traces of cysteic acid and glutathione were present on all chromatograms of the free amino acid fraction.

In both species the free amino compounds present in the highest concentrations were aspartic and glutamic acids, glutamine, serine, and alanine (Table V). These accounted for approximately 90% of the total weight of free amino compounds measured. Quantitative estimates of asparagine were not obtained in these analyses, but this amide was present in smaller amounts than glutamine, particularly in rusted tissue. It is of interest that proline, which is derived from glutamic acid, was present only in trace amounts in both species. Tryptophan was not detected in uninfected Khapli, but is known from other observations to be present in 'trace' amounts at least.

In the protein hydrolyzate of uninfected leaves (Table V) phenylalanine was present in the highest concentration, accounting for 20% of the amino acid content. Aspartic and glutamic acids, lysine, and the leucines each

TABLE IV

Protein amino acids (g/100 g fresh weight)

	Little Club								
	2 days			7 days			9 days		
	H	R	R/H	H	R	R/H	H	R	R/H
Dry weight	8.88	9.22	1.04	9.48	12.58	1.33	9.45	21.11	2.23
Aspartic	0.274	0.348	1.27	0.174	0.310	1.78	0.257	0.484	1.88
Glutamic	0.271	0.333	1.23	0.284	0.259	0.91	0.255	0.387	1.52
Lysine	0.304	0.361	1.19	0.342	0.324	0.95	0.112	0.183	1.63
Arginine	0.098	0.134	1.37	Tr.	0.134	–	0.264	0.406	1.54
Proline	0.095	0.114	1.20	0.113	0.139	1.23	0.115	0.308	2.67
Phenylalanine	0.639	0.972	1.52	0.539	0.776	1.44	0.261	0.777	2.98
Tyrosine	0.067	0.091	1.36	0.090	0.049	5.44	0.077	0.308	4.00
Serine	0.085	0.143	1.68	Tr.	0.143	–	0.159	0.189	1.19
Threonine	0.090	0.130	1.44	Tr.	0.109	–	0.090	0.136	1.51
Alanine	0.195	0.244	1.25	0.200	0.192	0.96	0.148	0.217	1.47
Glycine	0.142	0.175	1.23	0.148	0.109	0.74	0.114	0.162	1.42
Leucines	0.244	0.147	0.60	0.313	0.165	0.53	0.284	0.243	0.86
Valine	0.167	0.184	1.10	0.170	0.162	0.95	0.126	0.186	1.48
Total	2.671 (30.080)	3.376 (36.616)	1.26 (1.22)	2.373 (25.032)	2.871 (22.822)	1.21 (0.91)	2.262 (23.937)	3.986 (18.882)	1.76 (0.79)

	Khapli											
	2 days			3 days			5 days			8 days		
	H	R	R/H	H	R	R/H	H	R	R/H	H	R	R/H
Dry weight	9.89	10.22	1.03	9.25	9.78	1.06	10.65	11.12	1.04	10.59	10.59	1.00
Aspartic	0.308	0.308	1.00	0.285	0.293	1.03	0.285	0.276	0.97	0.326	0.273	0.84
Glutamic	0.300	0.267	0.89	0.244	0.239	0.98	0.282	0.244	0.87	0.374	0.206	0.55
Lysine	0.283	0.271	0.96	0.151	0.139	0.92	0.269	0.269	1.00	0.287	0.204	0.71
Arginine	0.165	0.186	1.13	0.156	0.136	0.87	0.176	0.123	0.70	0.261	0.154	0.59
Proline	0.080	0.090	1.13	0.089	0.126	1.42	0.129	0.116	0.90	0.093	0.105	1.13
Phenylalanine	0.577	0.320	0.55	0.645*	0.645*	1.00	0.734	0.323	0.44	0.277	0.137	0.49
Tyrosine	0.070	0.073	1.04	Tr.	Tr.	–	Tr.	Tr.	–	0.015	0.069	4.60
Serine	0.151	0.151	1.00	0.080	0.107	1.34	0.121	0.123	1.02	0.161	0.148	0.92
Threonine	0.134	0.120	0.90	0.101	0.082	0.81	0.085	0.095	1.12	0.124	0.093	0.75
Alanine	0.207	0.189	0.91	0.173	0.157	0.91	0.181	0.163	0.90	0.188	0.145	0.77
Glycine	0.150	0.130	0.87	0.109	0.128	1.17	0.113	0.127	1.12	0.161	0.159	0.99
Leucines	0.280	0.324	1.16	0.262	0.451*	1.72	0.300	0.451	1.50	0.277	0.247	0.89
Valine	0.141	0.130	0.92	0.156	0.129	0.83	0.168	0.147	0.88	0.142	0.096	0.68
Total	2.846 (28.776)	2.559 (25.079)	0.90 (0.87)	2.419 (26.151)	2.632 (26.914)	1.09 (1.03)	2.843 (26.694)	2.457 (22.095)	0.86 (0.83)	2.686 (25.364)	2.036 (19.226)	0.76 (0.76)

*Interpolated values. Samples lost.

NOTE: Figures in brackets are g/100 g dry weight. Tr. = trace.

TABLE V

	Mean concentrations of free amino acids				Mean concentrations of protein amino acids			
	Little Club		Khapli		Little Club		Khapli	
	g/100 g f.w.	%	g/100 g f.w.	%	g/100 g f.w.	%	g/100 g f.w.	%
Aspartic	0.0158	11.22	0.0178	12.91	0.235	9.6	0.301	11.1
Glutamic	0.0512	36.36	0.0539	39.09	0.270	11.0	0.300	11.0
Glutamine	0.0097	6.89	0.0090	6.53	—		—	
Lysine	0.0028	1.99	0.0002	0.15	0.253	10.3	0.248	9.1
Arginine	0.0025	1.78	0.0010	0.73	0.124	5.1	0.190	7.0
Proline	0.0020	1.42	0.0020	1.45	0.108	4.4	0.098	3.6
Phenylalanine	0.0026	1.85	0.0020	1.45	0.480	19.6	0.558	20.5
Tyrosine	0.0020	1.42	0.0020	1.45	0.078	3.2	0.031	1.1
Tryptophan	0.0020	1.42	—	—	—		—	
Serine	0.0254	18.04	0.0167	12.11	0.085	3.5	0.128	4.7
Threonine	0.0021	1.49	0.0020	1.45	0.063	2.6	0.111	4.1
Alanine	0.0158	11.22	0.0219	15.88	0.181	7.4	0.187	6.9
Glycine	0.0005	0.36	0.0035	2.21	0.135	5.5	0.133	4.9
Leucines	0.0034	2.41	0.0020	1.45	0.280	11.4	0.280	10.3
γ-Aminobutyric	0.0012	0.85	0.0028	2.03	—		—	
Valine	0.0018	1.28	0.0011	0.80	0.154	6.3	0.152	5.6
Total	0.1408	100	0.1379	100	2.446	100	2.717	100
Dry weight	9.27		10.10		9.27		10.10	

accounted for approximately 10%. Trace amounts of hydroxyproline were present in each species. It was not found in the free amino acid pool.

The most striking feature of these analyses is the over-all similarity in the concentrations and percentage compositions of the corresponding amino acid fractions in the two species of wheat. Equally striking are the differences in the percentage compositions of the free amino acid pool and the protein hydrolyzate in each species, the outstanding example being the difference in the concentrations of free and combined phenylalanine. It is of course well known that the percentage composition of the free amino acid pool does not reflect the composition of the bulk protein.

Finally, examination of Tables III and IV will show that the total concentrations of both free and protein amino acids in Little Club and of the protein amino acids in Khapli fell as the uninfected leaves aged. In Little Club particularly striking decreases occurred in the free basic, aromatic, and hydroxy-amino acids. In Khapli, the total concentration of free amino acids remained approximately constant. In both species the most striking changes in protein amino acids with age were a decrease in phenylalanine and a decrease in the ratio of lysine to arginine, without any marked alteration in the total concentration of these two basic amino acids.

Effects of Rust Infection on Amino Acid Levels

A. Free Amino Acids (Table III)

In infected leaves of Little Club the concentration of free amino acids rose steadily, and on a fresh weight basis was more than fourfold that in uninfected leaves at 9 days after inoculation. Relative to the concentrations in uninfected tissue, the most striking increases occurred in glutamine, γ-aminobutyric acid, threonine, and those amino acids present only in trace amounts before inoculation, particularly the basic and aromatic acids. Lesser increases occurred in aspartic and glutamic acids and alanine; those in glutamic acid and alanine did not keep pace with the increase in dry weight. Maximum concentrations of the different amino acids were reached at different times and the percentage composition of the free amino acid pool changed progressively throughout the course of rust development. The extent of such alterations is illustrated by the derived data in Table VI. Glutamic and aspartic acids and glutamine together accounted for 38, 49, and 56% of the amino acids estimated at 2, 7, and 9 days after inoculation, respectively. The relative concentrations of glutamate, glutamic acid, and γ-aminobutyrate shifted from approximately 22:4:1 at 2 days, through 9:7:1 at 7 days, to 6:7.5:1 at 9 days after inoculation. These results suggest the synthesis of glutamine (by amidation) and γ-aminobutyric acid (by decarboxylation) from glutamate. Proline, which can also arise from glutamate, did not accumulate in the free amino acid pool, but increased amounts of proline were found in the protein hydrolyzate (see below). Aspartic acid increased in concentration after infection and accounted for approximately 7, 13, and 16% of the total amino acids estimated at 2, 7, and 9 days after inoculation, respectively. At the same time the ratio of glutamic to aspartic acid fell progressively from 3.6 through 1.70 to 1.4. The results are consistent with the production of aspartate from glutamate by transamination to oxaloacetate.

TABLE VI

Percentage composition of free amino acid pool

	Little Club (9 days)			Khapli (8 days)		
	H	R	R−H	H	R	R−H
Aspartic	12.4	14.0	+1.6	12.0	12.9	+0.9
Glutamic	41.2	18.9	−22.3	36.1	25.7	−10.4
Glutamine	8.1	22.9	+14.8	7.5	15.9	+8.4
Lysine	0.2	4.5	+4.3	0.1	0.2	+0.1
Arginine	0.8	5.5	+4.7	0.7	0.8	+0.1
Proline	1.6	0.8	−0.8	1.3	1.6	+0.3
Phenylalanine	—	2.7	+2.7	0.1	3.3	+3.2
Tyrosine	1.6	1.9	+0.3	1.3	1.6	+0.3
Tryptophan	1.6	4.4	+2.8	—	0.3	+0.3
Serine	15.4	10.6	−4.8	19.8	6.8	−13.0
Threonine	0.8	2.1	+1.3	1.0	1.6	+0.6
Alanine	13.2	5.4	−7.8	12.3	15.4	+3.1
Glycine	0.6	0.7	+0.1	1.6	0.3	−1.3
Leucines	1.6	1.8	+0.2	1.3	4.1	+2.8
γ-Aminobutyric	0.8	3.1	+2.3	3.5	7.2	+3.7
Valine	0.2	1.4	+1.2	1.3	2.1	+0.8
Total	100	100		100	100	

In Khapli, the general level of the free amino acids fell after infection. Relative to the concentrations in healthy tissue, particularly striking decreases eventually occurred in glutamate, serine, threonine, glycine, and the leucines. Serine showed a marked transitory increase at 3 days after inoculation, while glutamine and γ-aminobutyric acid exhibited well-defined, steady increases. In infected tissue the relative concentrations of glutamate, glutamine, and γ-aminobutyric acid were approximately 11:3:1 at 3 days, 4:2:1 at 5 days, and 3.5:2:1 at 8 days after inoculation, so that the results were in this respect similar to those for Little Club. Finally, the percentage composition of the free amino acid pool was less affected by infection in Khapli than in Little Club (Table VI).

B. Protein Amino Acids (Table IV)

In infected Little Club the total concentration of protein amino acids per gram fresh weight rose to 1.76-fold that in uninfected tissue by 9 days after inoculation. Per gram dry weight this represented a relative loss of protein amounting to 20%. A similar loss occurred in Khapli whether the results were expressed on a fresh or a dry weight basis.

By comparison with the concentrations in uninfected tissue, the most striking increases in infected Little Club occurred in serine at 2 days, in aspartic at 7 days, and in proline, phenylalanine, and tyrosine at 9 days after inoculation. Similarly, in infected Khapli, relative to the concentrations in uninfected tissue, increases in proline and the leucines were observed at 3 days, in the leucines at 5 days, and in tyrosine at 8 days after inoculation. On the other hand, aspartic acid did not increase and the concentrations of glutamate and phenylalanine fell to approximately 50% of the corresponding levels for uninfected tissue.

A comparison of the effects of these drifts on the percentage composition of the bulk protein in Little Club and Khapli at 9 days and 8 days after

TABLE VII

Percentage composition of protein hydrolyzate

	Little Club (9 days)			Khapli (8 days)		
	H	R	R−H	H	R	R−H
Aspartic	11.4	12.1	+0.7	12.1	13.4	+1.3
Glutamic	11.3	9.7	−1.6	13.9	10.1	−3.8
Lysine	4.9	4.6	−0.3	10.7	10.0	−0.7
Arginine	11.7	10.2	−1.5	9.7	7.6	−2.1
Proline	5.1	7.7	+2.6	3.5	5.1	+1.6
Phenylalanine	11.5	19.5	+8.0	10.3	6.7	−3.6
Tyrosine	3.4	7.7	+4.3	0.5	3.4	+2.9
Serine	7.0	4.8	−2.2	6.0	7.3	+1.3
Threonine	4.0	3.4	−0.6	4.6	4.6	0.0
Alanine	6.6	5.5	−1.1	7.0	7.1	+0.1
Glycine	5.1	4.1	−1.0	6.0	7.8	+1.8
Leucines	12.5	6.1	−6.4	10.3	12.1	+1.8
Valine	5.6	4.7	−0.9	5.3	4.7	−0.6
Total	100	100		100	100	

inoculation, respectively, is given in Table VII. Here it is clearly shown that after infection the changes in phenylalanine, serine, and the neutral amino acids with the exception of valine occur in opposite directions in the two species of wheat.

Discussion

The dry weight of leaf disks (diameter 2.8 mm) of Little Club bearing rust infections was sometimes as much as 2.5-fold that of disks cut from uninfected leaves (Fig. 1). The percentage dry weight of the 'bulk' sample, which contains a higher proportion of uninvaded tissue, may also be doubled after infection. These figures do not include the weights of spores that had already matured, since these were removed before weighing; they therefore imply that, if the entire increase in weight is due to the fungus itself, the heavily infected leaf may nurture more than its own dry weight of rust mycelium. Infection with *Erysiphe* caused increases in the dry weight of the underlying tissue amounting to approximately 50% of the total increase in weight of leaf disks bearing infections (Table I). Argument by analogy is often misleading and there are obvious differences between the closely packed intercellular mycelium of the rust and the loose, ectoparasitic mycelium of the mildew fungus. Nevertheless, it is our opinion that a part of the massive increase in dry weight that occurs at rust infections on susceptible wheat leaves is to be attributed to an accumulation of dry matter in the host tissue itself. This may amount to less than 25% of the total gain in weight. (Compare Shaw and Samborski 1956 and Samborski and Shaw 1956.)

The main changes that occur in nitrogenous constituents at developing rust infections on resistant and susceptible species of wheat have been described in this investigation. To recapitulate, at infections on the susceptible species, Little Club, there is a striking increase in nitrogen content per gram fresh weight and an increase in the ratio of soluble to insoluble nitrogen (Figs. 1, 3, and 4). The detailed amino acid analyses (Tables III and IV)

show that this is accompanied by up to a fourfold increase in free amino acids but also by a net synthesis of protein, since the concentration of protein amino acids per gram fresh weight is nearly doubled. Since the dry weight per gram fresh weight is increased twofold or more, the gain in protein is obscured when the results are expressed on a dry weight basis. This indicates the concomitant accumulation of nonproteinaceous dry matter.

These results are consistent with the view that soluble N is translocated into the infected susceptible leaf and retained there (Samborski and Shaw 1956). They are also consistent with the observation (Shaw and Samborski 1956) that radioactive sugars, amino acids, and P^{32} are 'actively' accumulated at rust infections when fed to infected leaves. In arriving at this interpretation of the data the suggestion (D'Oliveira 1939) that rusts fix atmospheric nitrogen has been ignored, both because it has not been checked by the use of $(N^{15})_2$ and because of the magnitude of the changes reported here. Whether or not, and to what extent, fixation of atmospheric nitrogen occurs in infected tissue remain open questions.

The picture following infection of the resistant variety, Khapli, is completely different. Here, any increases in the concentration of nitrogenous fractions are slight, transitory, and occur very soon after infection (Figs. 2, 3, and 4). The infected leaf rapidly reaches a stage at which the concentrations of soluble and insoluble nitrogen and the ratios of soluble to insoluble nitrogen and free to protein amino acids all decline drastically, soluble nitrogen being transported to other parts of the plant.

The analyses also demonstrate clearly that, to a large extent, changes in the concentrations of individual amino acids occur independently, thus resulting in changes in the percentage composition of both free amino acid pool and the protein (Tables VI and VII).

In considering the composition of the free amino acid pool it must be remembered that this is made up of at least four components, namely the vacuolar and cytoplasmic 'pools' of both host and parasite, and that the compositions of these separate pools may differ markedly from one another though, of course, the data give no information as to the magnitude of such differences. For example, theoretically, an amide, say glutamine (see below), may accumulate in the host tissue at infection sites and yet be rapidly metabolized after being taken up by the fungus. Again, the haustoria of the fungus are in intimate contact with the cytoplasm of the host and the parasite may thus draw a supply of amino acids primarily from the cytoplasmic pool of the host, amino acids present in the vacuoles of host cells being relatively less available. These points, which are of theoretical interest, apply equally well to other soluble metabolites.

The most conspicuous effect of infection was to cause an accumulation of free glutamine (Tables III, IV, and VI). This may originate either from an hydrolysis of protein, or by an endergonic reaction, requiring ATP, from glutamate. In view of the net gain in protein, synthesis from glutamate is perhaps the more likely alternative in Little Club (see below). The central role of glutamate and glutamine in linking carbohydrate and nitrogen metabolism needs no emphasis here. It is of interest that glutamine is also accumulated in rust-infected sunflower leaves (unpublished observations) and, though

on a reduced scale, even at resistant infections on Khapli. Rohringer (1957) has already reported that uredospores are rich in glutamine and that this amide accumulates in rust-infected leaves of several other varieties of wheat.* He has also drawn attention to the fact that glutamine and hexose-6-phosphate are precursors of glucosamine, which, as *n*-acetylglucosamine, is the building unit of chitin. Bassett (1957) has reported that the concentration of *n*-acetylglucosamine released by hydrolysis with chitinase showed a sudden and pronounced increase in concentration in rust-infected leaves of a susceptible variety of wheat at a stage (10–13 days after inoculation in his experiment) when the respiration rate had already reached its peak. During the synthesis of chitin thus indicated, the concentration of reducing sugars fell to one-quarter its peak value. Smith (1959) has reported that the activity of DPN-specific (low activity with TPNH) glutamic acid dehydrogenase is enhanced after infection of susceptible sunflower cotyledons with *Puccinia helianthi*. A highly active glutamic dehydrogenase was also found in germinated uredospores of this fungus. This enzyme catalyzes the formation of glutamate from α-ketoglutarate. Glutamic acid decarboxylase, which catalyzes the production of γ-aminobutyrate, was also present in uninfected and rusted sunflower cotyledons, but not in uredospores of *P. helianthi*. The activity of this enzyme was depressed after rust infection of sunflower cotyledons (*P. helianthi*), Little Club wheat leaves (*P. graminis*), and, during the summer time, bean leaves (*Uromyces phaseoli*). During the winter the activity of the enzyme in rusted bean leaves was higher than in uninfected plants. When combination of the enzyme with an inhibitor was prevented by extraction in the presence of excess glutamate, the decreases in activity of the glutamic decarboxylase could be partially or completely overcome by addition of pyridoxal phosphate to the reaction system. Smith (1959) speculated that in a susceptible variety, rust infection induces a shift in pyridoxal phosphate from glutamic decarboxylase, which in uninfected tissue is saturated with the coenzyme, to glutamic–aspartic transaminase, which is not normally so saturated (see below).

More recently, Reisener and McConnell (1961) have studied the metabolism of valerate-1-C^{14} in spores of *P. graminis tritici*. These authors isolated and measured the specific activity of the free and protein amino acids. Glutamic and γ-aminobutyric acids and glutamine had about the same specific activity which was some sixfold that of alanine, which in turn was 2–3 times as active as threonine, serine, and glycine. Glycine was the least active acid examined. Unfortunately, the specific activity of aspartate was not determined. The pattern of labelling in free glutamate (high activity in C_1) found by Reisener and McConnell (1961) suggested that the valerate underwent extensive β-oxidation to acetyl CoA, which was subsequently metabolized via the tricarboxylic acid cycle. The protein amino acids had much lower specific activities than the free acids, but glutamic and aspartic were, respectively, the two most radioactive. The authors suggest that there may have been some synthesis of protein during the incubation period.

*Since this was written, R. Siebert (Phytopathol. Z. **40**, 221–244 (1961)) has reported that both glutamine and asparagine are increased in rust-infected wheat.

The absolute and relative changes in free glutamate, glutamine, and γ-aminobutyrate described on pages 1364–65 are consistent with the observations of Reisener and McConnell (1961) and Smith (1959), except, perhaps, for Smith's conclusion that glutamic decarboxylase activity is depressed after infection. The absolute and relative changes in glutamate and aspartate are also consistent with an increased activity of glutamate–aspartate transaminase. Smith (1959) has found an increased activity of this enzyme in rusted, susceptible sunflower tissue; on the other hand its activity in extracts of germinating uredospores of *P. helianthi* was very low.

All these results thus give credence to the view (Shaw 1959) that the tricarboxylic acid cycle operates in rust-infected tissue and that glutamate and glutamine play a crucial role in the metabolism of the host–parasite complex. It is also worthy of note that proline and arginine are both believed to be derived from glutamate. Arginine accumulated in the free amino acid pool in infected Little Club but proline did not (Table III). Reisener and McConnell (1961) found that proline and arginine did not become labelled when valerate-1-C^{14} was fed to incubated rust spores. It has already been pointed out (Shaw 1959) that the increase in CO_2 produced via the pentose phosphate pathway after infection (Shaw and Samborski 1957; Daly, Sayre, and Pazur 1957) is not necessarily incompatible with a simultaneous increase in the absolute rate of turnover of the tricarboxylic acid cycle, though of course the percentage contribution of the latter is lowered.

There is evidence (for references, see Neish 1960) that in fungi and higher plants, as in bacteria (Davis 1958), aromatic amino acids arise from erythrose-4-phosphate and phospho-enol pyruvate via pathways involving shikimic acid. Erythrose-4-phosphate is an intermediate in the pentose phosphate pathway, in which it arises from sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate in the transaldolase reaction. Neish (1960) has pointed out that production of erythrose-4-phosphate does not necessarily depend on the operation of complete respiratory or photosynthetic pentose phosphate cycles. Nevertheless, it is tempting to see a connection between the increased role of the pentose phosphate pathway in the respiration of the host–parasite complex (Shaw and Samborski 1957) and the increased levels of aromatic amino acids (Tables III and IV) and indole compounds, such as indoleacetic acid (Shaw and Hawkins 1958), that occur in the rust-infected tissue of a susceptible species.

The most optimistic interpretation of the changes in protein composition in infected Little Club (Tables IV and VII) is that they represent minimal differences between the protein of the leaf and that of the parasite, since the rust protein is 'diluted' by that of the leaf. This is, however, by no means certain and alterations in the composition of the leaf protein itself as a result of infection cannot be excluded. In Khapli, on the other hand, relatively little fungal mycelium develops at the type 1 infections, and accordingly it is safer to assume that in this species the changes in composition of the protein hydrolyzate (Tables IV and VII) chiefly reflect changes in the leaf protein. Judging from microscopic examination of chloroplast degeneration in infected cells, the loss of chloroplast protein is much more rapid and extensive in this resistant species. Nevertheless, it is clear that the net changes

in both the amount and the composition of the protein after infection are more extensive in the susceptible species, Little Club.

Barrett and McLaughlin (1954) have carried out a semiquantitative study of aqueous 'protein extracts' of uninfected and rusted (Race 9 of *P. tricticina*) plants of Pawnee (resistant) and Michigan Amber (susceptible) wheats. The extracts were examined electrophoretically and, after hydrolysis with hydrochloric acid, by paper chromatography. It was found that infection had no effect on the mobility or composition of the protein from Pawnee wheat (resistant). On the other hand the protein from Michigan Amber exhibited a significantly higher negative mobility after infection. This was attributed, on the basis of the chromatographic analysis, to an increase in carboxyl (aspartic and glutamic) and a decrease in amino (lysine) groups in the protein. It is noteworthy that their chromatographic evidence for an increase in carboxyl groups after infection was their failure to detect glutamic acid in Michigan Amber before infection and its appearance after infection. If this observation is in fact correct, and this seems unlikely, Michigan Amber differs from Little Club, Khapli, and the several varieties examined by Rohringer (1957) in all of which glutamic acid is an important component of the protein amino acids from uninfected leaves. It is also noteworthy that infected Michigan Amber and infected and uninfected Pawnee all contained approximately the same concentration of glutamic acid. Barrett and McLaughlin (1954) suggest that *P. tricticina* would attack wheats having proteins with a high ratio of amino to carboxyl groups and that the attack would result in a fall in this ratio. Rohringer's results give no support to this hypothesis and there is also no evidence in any of the data presented above to suggest an increase in the ratio of carboxyl to amino groups in the protein of Little Club after infection with stem rust.

The question now arises whether the results that have been presented have any bearing on the problem of the mechanism of resistance to rust. The analyses carried out for urea and ammonia give no support to the claim that resistance is associated with toxic levels of these substances. The formation of large amounts of glutamine could, however, be regarded as a detoxification mechanism for removal of ammonia. The amino acid composition of the leaf-protein of Little Club and Khapli leaves is strikingly similar (Table V). Results of this kind are not, of course, expected to give information about specific proteins which, if involved in resistance, may differ only in a few amino acid residues per molecule (cf. normal and sickle cell hemoglobin) (Ingram 1957).

Recently, Samborski and Forsyth (1960) have obtained some information on the importance of specific compounds to the wheat rust complex by the use of antimetabolites. Of the amino acid analogues tested, canavanine, ethionine, and *p*-fluorophenylalanine were the most effective in inhibiting rust development on detached leaves floated on dilute, unbuffered solutions of benzimidazole (30–40 p.p.m.) under nonsterile conditions. These inhibitions were prevented by arginine, methionine, and phenylalanine respectively. As mentioned earlier, methionine was not detected in any of the analyses reported in this paper, but this is presumably the result of technical inadequacies. The development of successful infections on Little Club was accom-

panied by increases in arginine and phenylalanine as well as tyrosine and tryptophan. Phenylalanine and tryptophan also accounted for an increased percentage of the protein amino acids after infection. Again, Samborski and Forsyth (1960) found that 0.2% serine prevented rust development. In the work reported here the highest ratio (6.0) of serine in rusted relative to uninfected tissue occurred at 3 days after inoculation of the resistant species, Khapli. Moreover, serine accounts for a decreased percentage of both free and protein amino acids in the later stages of rust development on Little Club. All these observations may be more than coincidental and give credence to the idea that the concentrations of individual amino acids relative to one another (Samborski and Forsyth 1960) and probably to other substances as well may affect rust development. The relative compositions of the free amino acid pools in uninfected Little Club and Khapli are remarkably similar (Table V).

Finally the results that have been obtained show that a more intensive study of amino acid and protein levels and turnover in infected plants grown under controlled conditions would be of considerable interest. It is also evident that results which have been obtained in different laboratories employing completely different techniques and different species of rust are strikingly complementary where they impinge upon one another.

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LABORATORY STUDIES ON MICROFUNGI ISOLATED FROM THE STEMS OF LIVING LODGEPOLE PINE, PINUS CONTORTA DOUGL.[1]

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Abstract

Fungus isolation attempts, made from samples taken at 2-ft intervals in the stems of 40 lodgepole pines (*Pinus contorta* Dougl.), gave 1362 cultures. Sample trees were located at two sites and averaged 82.5 and 63.9 years of age. In malt agar culture *Tympanis hypopodia* Nyl. and the *Coryne sarcoides* complex were slightly antagonistic to *Stereum pini* (Schleich. ex Fr.) Fr. Both microfungi caused small weight losses in pine wood blocks and, when inoculated in advance on test blocks, reduced the decay caused by *S. pini*. *Retinocyclus abietis* (Crouan) Groves & Wells caused no weight loss but increased the decay attributable to *S. pini*. *Tympanis hypopodia* and *C. sarcoides* complex were more or less restricted to the lower heartwood of the trees. There was some evidence of a succession of fungi in the trees.

Introduction

The importance of fungus succession in the decay of logging residue and insect-killed timber has been established (Chesters 1950; Mangenot 1954; Basham 1959). That similar phenomena might occur during the development of heart rot in living trees has been suggested (Mangenot 1954; Etheridge and Carmichael 1955). As an initial step in the examination of this hypothesis the occurrence of nonbasidiomycete fungi in the stems of living lodgepole pine has been investigated. Descriptions of the more frequently isolated fungi together with data on their incidence will be published elsewhere.

The role that microfungi play in heart rot development is unknown but three possibilities are: some may themselves cause appreciable deterioration of the heartwood; some may produce synergistic effects with other specific fungi, possibly wood-rotters; others may produce antagonistic effects with specific fungi. Thus, it may be that certain microfungi hasten the development of heart rot while others retard it. The laboratory experiments reported here were undertaken to study these possibilities.

Field Methods

Twenty trees were selected in each of two areas in Alberta with the random-pair, random-bearing technique (180° exclusion angle) described by Cottam and Curtis (1949). The ages of the 20 sample trees at Kananaskis ranged from 51 to 79 years (av. 63.9 years) and those at Strachan from 71 to 91 years (av. 82.5 years). The selected trees were felled and bucked into 1-ft lengths. The first and subsequent alternate lengths were selected and a 1-in. disk cut from the basal end of each. These disks were numbered and placed immediately in a

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large plastic bag. Disks and blocks were then taken at once to the laboratory.

The blocks were split and cultures were attempted from the mid-portion of each, two from the pith, two from the heartwood, and two from the sapwood. Small chisels were used to isolate from the pith. A Swedish increment hammer (Hubert 1954) proved equally effective and more rapid for making isolations from the heartwood and sapwood. Culturing was done on 2% malt agar slants. Isolation attempts were completed within 5 hours of the time the tree was cut.

The disks were used for moisture determinations. Small samples, approximately 1 in. on a side, were cut with a chisel, one each from the pith region, the heartwood, and the sapwood. These were weighed to the nearest 0.01 g, dried at 105° C for 24 hours, and reweighed. The difference in weights when expressed as a percentage of the oven-dried weight gave an approximate percentage moisture content.

Wood-Decay Capabilities of Microfungi Isolated from Stems of Living Lodgepole Pine

To test their wood-decaying capabilities nine of the more frequently isolated microfungi (Table I) were grown on steamed lodgepole pine blocks. Loss in weight was the criterion of decay.

TABLE I

Microfungi isolated from stems of living lodgepole pine at Kananaskis and Strachan, Alberta

Fungus	Accession No. of typical isolate in DAOM*	Number of isolates		Percentage of trees infected	
		S†	K‡	S	K
Tympanis hypopodia Nyl.	59804	136	42	70	40
Coryne sarcoides complex	59806	67	14	35	5
Fumago-like fungus	59815	32	25	55	40
Zythia resinae (Ehrenb.) Karst.	59805	37	0	75	0
Aureobasidium pullulans (de Bary) Arnaud	59801	26	6	70	20
Taeniolella sp.	59802	24	8	70	35
Cladosporium herbarum (Pers.) Link	59803	22	6	45	15
Retinocyclus abietis (Crouan) Groves & Wells	59796	28	0	75	0
Hormodendrum sp.	59818	11	17	40	35
Cephalosporium sp.	59811	9	3	15	5
Heterobasidion-like fungus	59799	6	4	25	10
Tympanis sp.	59813	8	0	20	0
Paecilomyces varioti Bainier	59798	8	1	20	5
Septonema chaetospira (Grove) Hughes var.	59800	8	0	20	0
Penicillium frequentans Westling	Nil	4	0	20	0

*Mycological Herbarium, Department of Agriculture, Ottawa.
†Strachan.
‡Kananaskis.

Experimental Methods

Test blocks were cut from the heartwood of sample bolts that had given negative results in the original isolation attempts, and had since been air-dried for 12 months. The blocks were cut 1×1×0.25 in. with the tracheids running parallel to the short dimension. They were assigned to a series of decay chambers by using a table of random numbers.

Initial moisture-equilibrium weights for each block were determined under controlled temperature–humidity conditions. The blocks were air-dried and then placed in desiccators containing dilute sulphuric acid of specific gravity 1.5. Acid dilution during equilibration was controlled by making periodic specific gravity determinations, and adjusting the acid concentration as required. A relative humidity of 18% at 20° C was maintained in the desiccators, similar to relative humidities in the laboratory during most of this work. Thirty blocks were placed in each of 10 desiccators which were kept in constant-temperature cabinets at 20° C. Daily check weighings were made on two blocks from each desiccator. When there was no change in weight to the nearest 0.01 g for two successive days, all the blocks were weighed. The third decimal place was carried for computational purposes. Blocks were exposed to room atmosphere for 10 to 15 seconds while being weighed; little or no change in weight was recorded during the trial 15-second exposures to laboratory air. The test blocks were assumed to be free of fungi but, as an additional precaution, were steamed for 3 hours at atmospheric pressure and at 97° C prior to placement in the decay chambers.

Square-sided, 8-oz bottles were used as decay chambers (Zabel 1948). Approximately 25 ml of 2% malt agar was placed in each bottle and allowed to solidify with the bottle in a horizontal position. Two representative stocks of each microfungus were selected, one from Strachan and one from Kananaskis. Each bottle was inoculated in three places with one of the two stocks of the microfungi used. Ten chambers were left uninoculated to serve as controls. One week after inoculation, sterile, V-shaped pieces of glass rod, 3 mm in diameter, were placed flat on the surface of the agar in such a way that they partially surrounded each developing fungus colony. Three steamed wood blocks were then placed aseptically in each chamber, one on each glass support. The experiment was replicated five times, involving, with the 10 control chambers, a total of 100 decay chambers.

After 3 months' incubation in the dark at 25° C, one block was removed from each chamber. These blocks were placed in desiccators, and allowed to come to moisture-content equilibrium at 20° C over sulphuric acid of specific gravity 1.5, and then weighed. The remaining two blocks in each chamber were used in a subsequent experiment designed to measure decay caused by *Stereum pini* (Schleich. ex Fr.) Fr. in test blocks previously infected by microfungi.

Results

The weight loss data are presented in Table II. These are expressed as percentages of the original moisture-equilibrium weights, which were about 4% above oven-dry weights. The positive values can be explained by experimental error or the possibility that the fungi, drawing nutrient from the malt agar, produced enough mycelium to account for some increase in weight.

Wood Decay by *Stereum pini* using Test Blocks Previously Infected by Microfungi

Experimental evidence was sought on the occurrence of fungus interactions by inoculating wood blocks with microfungi and then, after 3 months, with *Stereum pini*.

TABLE II

Weight loss of pine heartwood test blocks exposed to two stocks of nine microfungi for 3 months

Fungus	Stock No.	Mean weight loss*
Control	—	+0.1
Tympanis hypopodia Nyl.	1	−0.5†
	2	−0.5†
Coryne sarcoides complex	1	−0.6‡
	2	−0.3
Aureobasidium pullulans (de Bary) Arnaud	1	−0.1
	2	−0.5‡
Zythia resinae (Ehrenb.) Karst.	1	+0.2
	2	0.0
Fumago-like fungus	1	+0.2
	2	+0.2
Retinocyclus abietis (Crouan) Groves & Wells	1	+0.2
	2	+0.2
Taeniolella sp.	1	+0.2
	2	+0.2
Hormodendrum sp.	1	+0.1
	2	+0.2
Cladosporium herbarum (Pers.) Link	1	0.0
	2	0.0

*Expressed as percentage of initial moisture-equilibrium weight.
†Significant at the 1% level when compared with controls.
‡Significant at the 5% level when compared with controls.

Experimental Methods

One hundred and ninety-five decay chambers were used, each containing malt agar and one V-shaped glass rod. Ninety-five chambers were inoculated with stock 1 and another 95 with stock 2 of *S. pini*. The five remaining chambers were left uninoculated to serve as controls. After the colonies of *S. pini* had developed for 1 week, one-half of the infected blocks remaining in the original chambers (one from each) were placed in the chambers containing stock 1 of *S. pini* (one to a chamber). The other blocks from the original decay chambers were similarly placed in the chambers containing stock 2 of *S. pini*. Uninfected control blocks from the chambers used in the experiment previously described were placed, one each, in the uninoculated control chambers. After 3 months' incubation in the dark at 25° C, the blocks were removed from the decay chambers and allowed to come to moisture equilibrium as in the previous experiment. Statistical comparisons were made with the *t* test.

Results

Weight losses of the blocks, expressed as percentages of the original moisture-equilibrium weights, are presented in Table III. The two stocks of *S. pini* showed a statistically significant difference in their ability to produce decay; stock 1 caused an average weight loss of 10.1% for the five replicates, and stock 2 an average weight loss of 3.6%.

The two isolates of *S. pini* also showed differences in their reaction to the microfungi established previously in the test blocks. Wood blocks that were attacked initially by either stock of *Retinocyclus abietis* (Crouan) Groves & Wells were decayed by stock 1 of *S. pini* to about the same extent as the blocks

that were free of microfungi (Table III). Stock 2 of *S. pini* behaved very differently. Wood blocks that were attacked earlier by either stock of *R. abietis* were decayed about three times as much by stock 2 as the blocks that were free of microfungi. This difference was statistically significant (Table III).

TABLE III

Effect of prior colonization by microfungi on subsequent attack of *Stereum pini* on steamed pine blocks

Fungus previously established in the blocks	Stock No.	Decay weight loss as percentage of initial weight	
		S. pini stock 1	*S. pini* stock 2
None (control blocks)	—	10.1	3.6
Retinocyclus abietis (Crouan) Groves & Wells	1	11.9	10.7*
	2	11.2	11.7†
Coryne sarcoides complex	1	7.0†	2.5
	2	5.0†	4.0
Taeniolella sp.	1	9.3	4.4
	2	10.1	3.1
Fumago-like fungus	1	7.6	4.4
	2	8.0	2.1
Tympanis hypopodia Nyl.	1	5.7†	2.6
	2	6.5	2.6
Cladosporium herbarum (Pers.) Link	1	8.0	2.2
	2	7.9	2.9
Aureobasidium pullulans (de Bary) Arnaud	1	9.0	5.7
	2	6.9	2.9
Zythia resinae (Ehrenb.) Karst.	1	12.0	3.5
	2	8.6	4.8
Hormodendrum sp.	1	10.1	3.3
	2	6.1	3.4

*Significant at the 1% level when compared with the controls.
†Significant at the 5% level when compared with the controls.

Wood blocks that were attacked first by either stock of the *Coryne sarcoides* complex were decayed less by stock 1 of *S. pini* than the blocks that were free of microfungi. This difference was statistically significant (Table III).

Wood blocks attacked previously by either stock of *Tympanis hypopodia* Nyl. were decayed less by either stock of *S. pini* than the blocks that were free of microfungi. The difference for *T. hypopodia* stock 1 combined with *S. pini* stock 1 was statistically significant; it was not significant for the other combinations (Table III).

The retardation of *S. pini* attack as a result of prior infection by *T. hypopodia* and the *C. sarcoides* complex was observable in the decay chambers. The mycelium of *S. pini* took much longer to cover the blocks previously infected with these fungi than to cover blocks infected with other fungi or the controls.

The remaining microfungi had no significant effect on subsequent attack by *S. pini* (Table III).

Antagonism in Plate Culture between Nine Microfungi and *Stereum pini*

Further evidence on the occurrence of interaction between the nine microfungi and *Stereum pini* was sought using malt agar media.

Experimental Methods

Stereum pini and the first nine fungi in Table I were used. It was assumed that variability would be less on a uniform agar substrate than on wood. Accordingly, the experiment was replicated twice instead of five times, permitting a test of four stocks of the fungi instead of two as in the previous experiment. A total of 288 pairings were made.

Each of the four stocks of the nine microfungi was paired with each of the four *S. pini* stocks by inoculating opposite edges of malt agar plates. After incubation for 1 week under room light and temperature conditions (about 22° C), the plates were observed every other day to note any antagonistic reactions. Observations were continued until one of the fungi had, to a large extent, overgrown the other.

Results

No examples of strong antagonism, as shown by the formation of clear inhibition zones, were recorded. The imperfect states of *Retinocyclus abietis*, *Tympanis hypopodia*, and the *Coryne sarcoides* complex were slightly antagonistic to *S. pini*; however, in all these experiments the latter fungus eventually grew over the other fungi.

The strongest reaction occurred in pairings of *S. pini* with the *C. sarcoides* complex (Fig. 1). Colonies of the *C. sarcoides* complex were almost completely surrounded by mycelium of *S. pini* before the latter made any growth over them. No obvious variation in the reactions of the different stocks of the fungi were observed.

Reactions between *R. abietis* and *S. pini* were more variable. They ranged from instances of very slight antagonism, where *S. pini* was checked briefly in its growth over *R. abietis*, to examples of moderate antagonism, in which *R. abietis* colonies were approximately half surrounded by mycelium of *S. pini* before the latter grew over them (Fig. 2). No apparent differences were noted between the two replicates.

A very mild type of antagonism was observed between *T. hypopodia* and *S. pini*. Reactions were similar between the different stocks of these fungi and there was no recognizable difference between the two replicates. The growth of *S. pini* stopped briefly on contact with the *T. hypopodia* colonies but it was quickly resumed, and the mycelium of *S. pini* grew over the other fungus (Fig. 3).

Pairings between the remaining six microfungi and *S. pini* revealed no apparent reactions. Mycelium of *S. pini* grew virtually unchecked over the colonies of the other fungi.

Histological

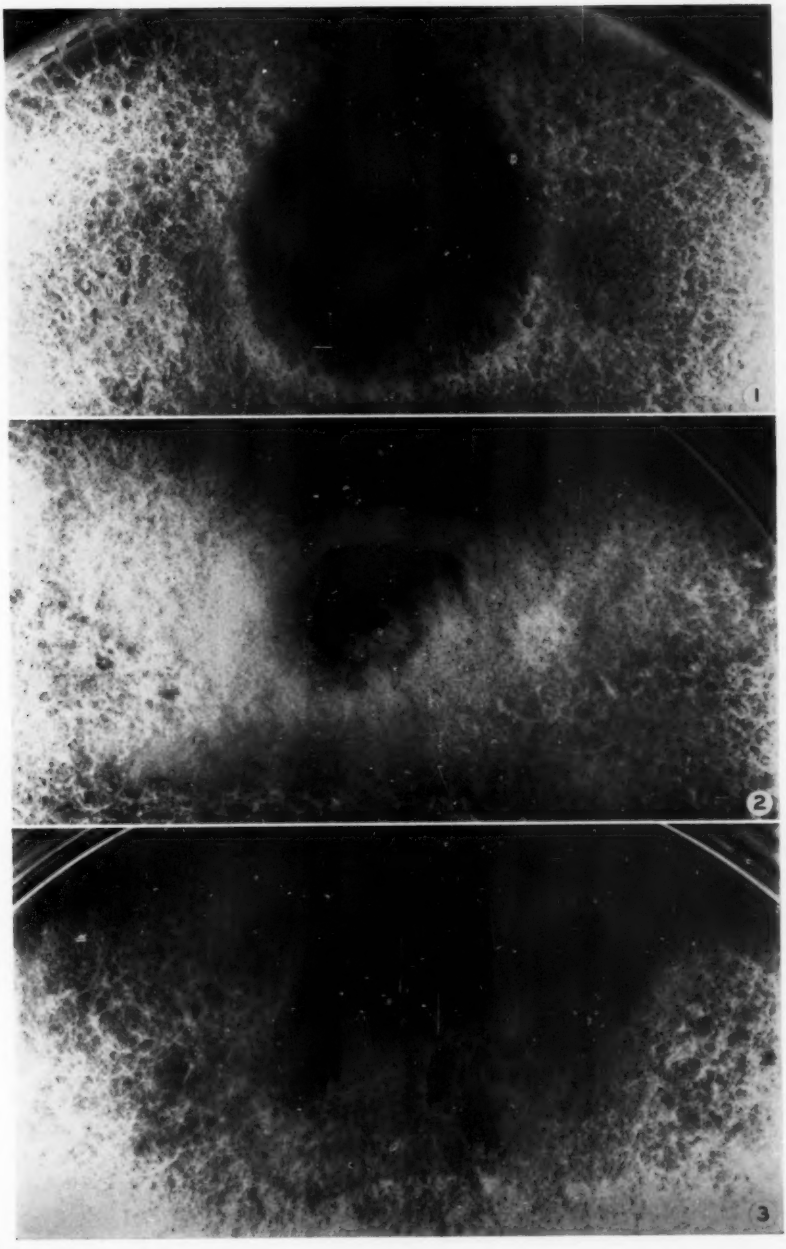
Naturally infected wood and inoculated wood were examined to determine whether each fungus inhabited similar tissues in both circumstances.

FIG. 1. Typical antagonistic reaction in plate culture between *Coryne sarcoides* complex and *Stereum pini*. About 2.5×.

FIG. 2. Typical antagonistic reaction in plate culture between *Retinocyclus abietis* and *Stereum pini*. About 2.5×.

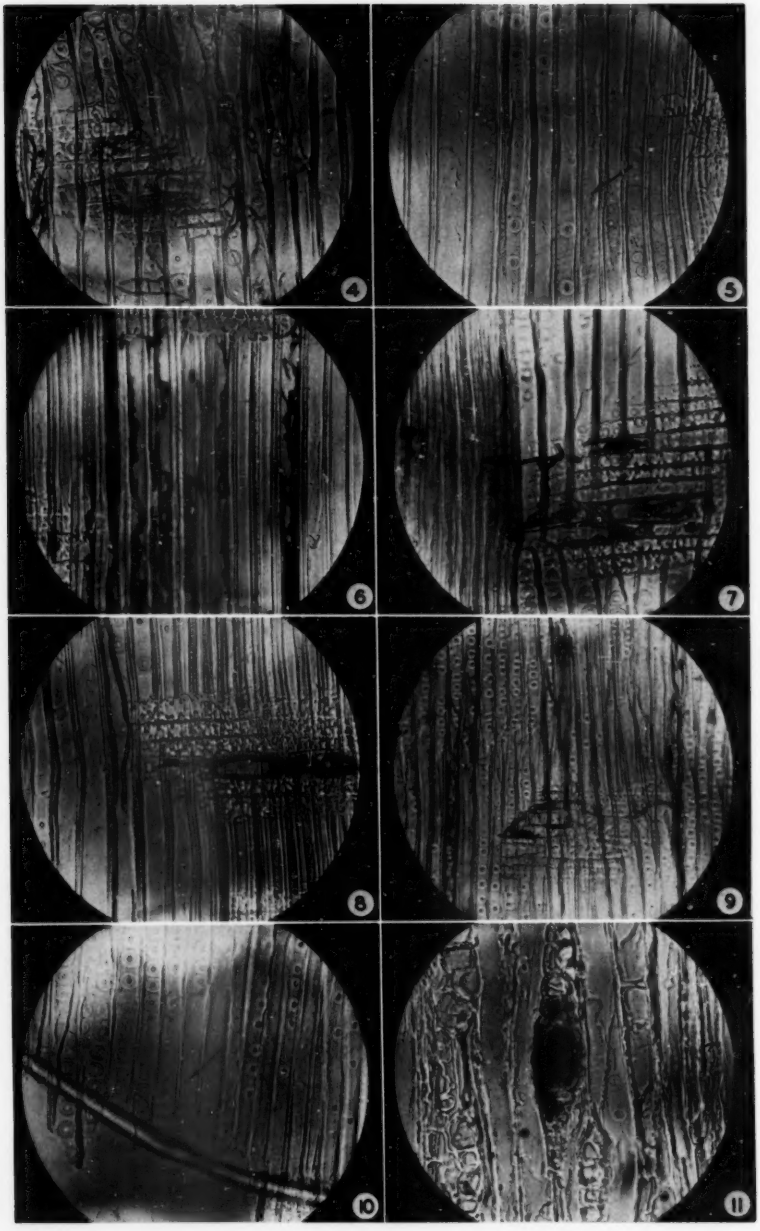
FIG. 3. Typical antagonistic reaction in plate culture between *Tympanis hypopodia* and *Stereum pini*. About 2.5×.

PLATE I



Bourchier—Can. J. Botany

PLATE II



Bourchier—Can. J. Botany

Experimental Methods

One-quarter to one-half inch cubes were cut from tree sections that had yielded the various fungi in culture. Air was removed by boiling the blocks in water until they sank. Radial and tangential sections were cut 15 to 20 μ thick with a sliding microtome. A few supplementary sections were cut free-hand using a razor blade for convenience. Sections made from naturally infected wood were stained with safranin and picroaniline blue and mounted in Canada balsam. Both conventional and phase-contrast illumination were used in the microscopic examination of the stained sections. Phase-contrast microscopy was soon found to be satisfactory for examining hyphae in wood and the staining procedure was discontinued for sections cut from inoculated blocks. These were left unstained and were mounted, directly from water, in Gurr's[3] aqueous mounting medium.

Results

Hyphae with typical basidiomycetous clamp connections were abundant in sections cut from blocks naturally infected with *Stereum pini*. The hyphae were observed to have occasionally penetrated the tracheid walls through bore holes of the enlarged type. Fine, simple-septate hyphae were abundant in sections cut from blocks naturally infected with *Cephalosporium* sp., but were scarce in those cut from blocks naturally infected with *Tympanis hypopodia*, *Paecilomyces varioti* Bainier, and *Penicillium frequentans* Westling. The hyphae of these microfungi were noted in the pits and lumina of the tracheids. No hyphae were found in blocks naturally infected with the remaining fungi (Table I). Resin ducts were frequently disorganized in this material.

Hyphae were found consistently, and often abundantly, in blocks inoculated with the nine microfungi, and in those inoculated with *S. pini*. Cultures of five species of fungi produced hyphae in the inoculated wood similar to those seen in naturally infected wood. Similar tissues were invaded under natural and artificial conditions by four of the fungi. Eleven microfungi were neither observed in naturally infected wood nor were they tested artificially.

Fine, simple-septate hyphae were observed in the pits and lumina of the tracheids and in the rays of blocks inoculated with *Hormodendrum* sp. (Fig. 4) and *Zythia resinae* (Ehrenb.) Karst. (Fig. 5). Dark, simple-septate hyphae were

[3]Obtained from George T. Gurr, Ltd., London, S.W. 6, England.

FIG. 4. Hyphae of *Hormodendrum* sp. in artificially infected lodgepole pine wood. About 200×.

FIG. 5. Hyphae of *Zythia resinae* in artificially infected lodgepole pine wood. About 200×.

FIG. 6. Hyphae of the *Fumago*-like fungus in artificially infected lodgepole pine wood. About 200×.

FIG. 7. Hyphae of *Aureobasidium pullulans* in artificially infected lodgepole pine wood. About 200×.

FIG. 8. Hyphae of *Cladosporium herbarum* in artificially infected lodgepole pine wood. About 200×.

FIG. 9. Hyphae of *Taeniolella* sp. in artificially infected lodgepole pine wood. About 120×.

FIG. 10. Hyphae of the *Coryne sarcoides* complex penetrating the tracheid wall in artificially infected lodgepole pine wood. About 160×.

FIG. 11. Hyphae of *Tympanis hypopodia* in a horizontal resin duct of artificially infected lodgepole pine wood. About 250×.

observed frequently in the ray parenchyma and also in the pits and lumina of the tracheids of blocks inoculated with the *Fumago*-like fungus (Fig. 6), *Aureobasidium pullulans* (de Bary) Arnaud (Fig. 7), *Cladosporium herbarum* (Pers.) Link (Fig. 8), and *Taeniolella* sp. (Fig. 9). Fine, simple-septate hyphae were observed penetrating the tracheid walls in blocks inoculated with the *Coryne sarcoides* complex (Fig. 10). Similar hyphae were observed concentrated in the resin ducts of blocks inoculated with *Tympanis hypopodia* (Fig. 11), and *R. abietis*.

As in naturally infected wood, hyphae were found consistently in the blocks inoculated with *S. pini*. In those blocks which had been inoculated with the microfungi prior to inoculation with *S. pini*, hyphae with clamps and hyphae typical of the other fungi as well were observed.

Blocks that were initially inoculated with fungi that showed some antagonism to *S. pini* in plate culture had far fewer hyphae with clamp connections (presumably of *S. pini*) than the other blocks.

Distribution of the Fungi in the Trees

Preliminary examination of the fungus isolation data indicated that some species were isolated more frequently from certain portions of the stems. In addition, certain of the fungi appeared to occur more frequently in or near red-stained wood. The distribution pattern of the fungi was explored further.

Experimental Methods

The data recorded when the cultures were made included information on the location of each isolation point in the tree. Information on the appearance of the wood, whether apparently normal or red-stained, was also recorded at that time. For the present analysis, the isolation points for each of the fungi were classified as being in the pith, heartwood, or sapwood, and as being in the lower, middle, or upper one-third of the bole. A fungus isolation point was considered associated with red-stained wood if it occurred within 2 ft of such material.

Results

The distribution of the microfungi in the trees is presented in Table IV. *Tympanis hypopodia* and the *Coryne sarcoides* complex both tended to be restricted to the lower-interior portions of the stem. All other microfungi appeared to be less specific in the portion of the stem inhabited.

All of the microfungi studied (Table I) occurred in apparently normal wood and were also associated with red-stained portions of the stems (Table V). *T. hypopodia* occurred more frequently in association with red-stained wood than in apparently normal wood. No well-defined trends were revealed for the other fungi, although *Taeniolella* sp., *Cladosporium herbarum*, and the *Coryne sarcoides* complex were somewhat more frequent in apparently normal wood.

Distribution of the Fungi as Influenced by Tree Age and Dominance

The presence of two age classes in the trees sampled for this study provided an opportunity to compare the incidence of the various fungi in trees averag-

TABLE IV

Distribution of the microfungi in 40 living lodgepole pine at Strachan and Kananaskis, Alberta

Fungus	No. of isolates					
	Radial			Vertical		
	In pith	In heartwood	In sapwood	In lower one-third	In middle one-third	In upper one-third
Tympanis hypopodia Nyl.	95	79	4	108	61	9
Coryne sarcoides complex	43	37	1	81	0	0
Fumago-like fungus	20	15	22	20	17	20
Zythia resinae (Ehrenb.) Karst.	19	9	9	12	14	11
Aureobasidium pullulans (de Bary) Arnaud	16	3	13	10	14	8
Taeniolella sp.	12	5	15	12	10	10
Cladosporium herbarum (Pers.) Link	10	7	11	10	6	12
Hormodendrum sp.	10	8	10	12	9	7
Retinocyclus abietis (Crouan) Groves & Wells	13	5	9	10	6	11
Cephalosporium sp.	5	3	4	8	2	2
Tympanis sp.	4	4	0	4	3	1
Heterobasidion-like fungus	3	2	5	4	4	2
Paecilomyces varioti Bainier	4	2	3	6	3	0
Septonema chaetospira (Grove) Hughes var.	2	2	4	5	1	2
Penicillium frequentans Westling	1	1	2	0	2	2

ing 82.5 and 63.9 years of age. It was realized that age was only one of many factors involved in this comparison.

TABLE V

Microfungi isolated from stems of living lodgepole pine at Kananaskis and Strachan, Alberta

Fungus	Percentage of culture attempts yielding the fungi*			
	In sound wood		Associated with red stain†	
	S‡	K§	S	K
Tympanis hypopodia Nyl.	1.2	1.7	7.0	4.7
Coryne sarcoides complex	2.7	0.9	1.8	0.0
Fumago-like fungus	1.8	1.4	0.5	1.2
Zythia resinae (Ehrenb.) Karst.	1.5	0.0	1.0	0.0
Aureobasidium pullulans (de Bary) Arnaud	1.5	0.2	0.3	0.8
Taeniolella sp.	1.5	0.5	0.2	0.0
Cladosporium herbarum (Pers.) Link	1.1	0.4	0.4	0.0
Retinocyclus abietis (Crouan) Groves & Wells	1.0	0.0	0.9	0.0
Hormodendrum sp.	0.3	0.7	0.4	2.0
Cephalosporium sp.	0.2	0.2	0.3	0.0
Heterobasidion-like fungus	0.2	0.2	0.2	0.4
Tympanis sp.	0.1	0.0	0.4	0.0
Paecilomyces varioti Bainier	0.2	0.1	0.3	0.0
Septonema chaetospira (Grove) Hughes var.	0.2	0.0	0.3	0.0
Penicillium frequentans Westling	0.1	0.0	0.1	0.0

*Percentage calculated on the total number of culture attempts made in sound wood and the total number made in wood associated with red stain.
†Associated with red stain, that is, either in red-stained wood or within 2 ft of it.
‡Strachan.
§Kananaskis.

Methods

Comparisons were based on the number of successful isolates in each class expressed as a percentage of the total number of culture attempts made in each class. The effect of tree age on the incidence of the fungi was determined by listing the occurrence of the fungi in each stand. The effect of tree dominance on the incidence of the various fungi was investigated by dividing each of the Strachan and Kananaskis samples into dominant and suppressed trees.

Results

Fungus incidence was higher in the older trees. The *t* test revealed that these differences were all highly significant (Table VI).

The incidence of the fungi in the dominant and suppressed trees is presented in Table VII. At Strachan, the percentage of isolation attempts yielding *S. pini* was significantly different (*t* test) in the dominant and suppressed trees. This was also true for the percentage of isolation attempts yielding the *Coryne sarcoides* complex. *Stereum pini* appeared about twice as often from suppressed trees as from dominant trees; all of the sample trees at Strachan, however, were infected with *S. pini* at some place in the stem. The *C. sarcoides* complex appeared about 10 times as often from dominant trees as from suppressed trees.

At the Kananaskis Forest Experiment Station similar trends were noted, but

the differences were not significant. The frequency of isolation of the remaining microfungi at either location was similar in the dominant and suppressed trees.

TABLE VI

Incidence of the microfungi and of *Stereum pini* in two lodgepole pine stands of different ages

Fungus	Average stand age: 82.5 years (Strachan)	63.9 years (Kananaskis)
All fungi	31.6*	9.2
Stereum pini (Schleich. ex Fr.) Fr.	17.4	2.3
Microfungi	13.8	6.9

*Number of successful cultures expressed as a percentage of the total number of culture attempts in that group of trees.

TABLE VII

Incidence of the microfungi and of *Stereum pini* in dominant and suppressed lodgepole pine at two locations

Fungus	Strachan		Kananaskis	
	Dominant	Suppressed	Dominant	Suppressed
All fungi	29.8*	42.5	9.4	13.7
Stereum pini (Schleich. ex Fr.) Fr.	12.4	23.5	2.2	7.3
Coryne sarcoides complex	3.3	0.3	1.3	0.0
All other microfungi	11.2	12.2	6.1	6.3

*Number of successful isolates expressed as a percentage of the total number of culture attempts in that group of trees.

Discussion and Conclusions

Normal-appearing heartwood of living lodgepole pine may support a rather constant flora of microfungi. Decay-capability tests of wood-rot fungi have been conducted frequently in the past with wood judged sound by visual examination. The present research indicates that close attention must be given to the microbiological condition of wood used for decay tests. Some of the variability encountered in past work might be explained by the presence or effects of microfungi in the test blocks.

Most of the microfungi did not cause appreciable weight losses in wood blocks. The most frequently isolated species, *Tympanis hypopodia* and the *Coryne sarcoides* complex, caused small, but statistically significant, weight losses. The data on the decay capabilities of the microfungi showed variability between replicates and between stocks of the fungi. Differences in the wood and possible strain differences in the fungi may have been involved. Part of the variability may have resulted from differences in the amount of aerial hyphae produced by the fungi. Those with raised hyphal mats contacted the test blocks early in the incubation period; others with appressed hyphal mats made contact after a longer delay.

The microfungi isolated in this study can be segregated into four groups: (1) resin-inhabiting fungi; (2) fungi capable of penetrating tracheid walls; (3) ray-parenchyma-inhabiting fungi; (4) other fungi. The last two groups

apparently have little importance in the development of heart rot.

Hyphae of the resin inhabiters, *T. hypopodia* and *Retinocyclus abietis*, were found concentrated in the resin ducts of inoculated test blocks. Resin ducts in naturally infected wood were frequently disorganized but hyphae were not observed. Possible shrinkage of the resin as a result of air-drying the blocks for 12 months may have destroyed the hyphae. *Tympanis hypopodia* occurred usually in the pith and heartwood of the lower regions of lodgepole pine stems. Moisture did not appear to be an important factor in this distribution; the fungus tolerated a moisture content as low as 23% in heartwood and as high as 143% in sapwood. *Tympanis hypopodia* may be either a root-inhabiting organism that moves up into the lower stem, or there may be a greater resin content in the lower heartwood of lodgepole pine favoring the occurrence of the fungus in that region.

The *Coryne sarcoides* complex is the only microfungus of wood-destroying capability isolated in this study. This organism caused small weight losses in test blocks, and hyphae were observed penetrating tracheid walls. The fungus was restricted more definitely than *T. hypopodia* to the heartwood and pith. It was isolated from wood that varied in moisture content from 30.5% to 79.1% of oven-dry weight. Sapwood had a moisture content of about 100% in the trees sampled; this fact might explain the almost complete exclusion of the *C. sarcoides* complex from the sapwood. The one isolation point in the sapwood from which this complex was obtained in culture had an unusually low moisture content of 54%, well within the apparent tolerance of the fungus.

Although the weight losses *in vitro* attributable to the microfungi were small or nonexistent, these fungi may cause undetermined changes in the wood which favor the subsequent establishment of typical heart-rot fungi, for instance, *Stereum pini*. All of the microfungi isolated in this study were obtained to some extent from unstained, apparently normal wood. Certain microfungi, however, were isolated more often from, or near, red-stained wood infected with *S. pini* than from apparently normal wood; it would appear that infection by microfungi may have preceded that by *S. pini* in these instances. The occurrence of a succession of fungi is therefore indicated.

Three of the microfungi, *R. abietis*, *T. hypopodia*, and the *C. sarcoides* complex, had a significant effect on the rate of decay of the wood by *S. pini*. *Retinocyclus abietis* gave a synergistic effect. Wood blocks, attacked initially by this fungus, were decayed significantly more by stock 2 of *S. pini* than the control blocks. The removal of resin by *R. abietis* may have increased the susceptibility of the wood to attack by *S. pini*.

Tympanis hypopodia and the *C. sarcoides* complex, when inoculated initially on wood blocks, had an inhibitory effect on subsequent decay by *S. pini*. Both these microfungi were slightly antagonistic to *S. pini* in Petri plates. The histological data gave further evidence of antagonism; hyphae with clamp connections, presumably of *S. pini*, were scarcer in wood blocks previously attacked by *T. hypopodia* and the *C. sarcoides* complex than in blocks that were free of microfungi.

The less frequent occurrence of the *C. sarcoides* complex in the suppressed trees may explain why *S. pini* was more common in those trees. Other factors

that might have contributed to the higher rate of infection and spread of *S. pini* in the suppressed trees were not discovered.

The experimental work indicates that there are interactions of various kinds between these fungi. Further research is needed to clarify the interrelationships of the microfungi with one another and with *S. pini* in lodgepole pine stems. The sampling of still younger trees might aid in determining the order in which fungi colonize the trees.

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SEROLOGICAL PROPERTIES COMMON TO DIFFERENT ISOLATES OF SOME STONE-FRUIT VIRUSES[1]

J. H. TREMAINE AND R. S. WILLISON

Abstract

Virus entities in cucumber associated with cherry yellows, green ring mottle, and necrotic ring spot of sour cherry; tatter leaf of sweet cherry; and prune dwarf were found to be serologically related but not identical. The virus antigens were compared in gel-diffusion precipitin tests with antisera prepared from both cucumber leaves and infected cherry petals.

Introduction

The cherry-yellows group of stone-fruit viruses has been shown to be associated with at least five distinct diseases: cherry yellows, green ring mottle, and necrotic ring spot of sour cherry; tatter leaf of sweet cherry; and prune dwarf. At this laboratory, virus entities associated with these diseases were mechanically transmitted to cucumber and compared with one another in studies of thermal inactivation, longevity *in vitro*, and dilution end point (2, 3, 4). A preparative technique, reported previously (6), was found to yield viable preparations of cherry yellows and necrotic ring spot virus isolates suitable for studies of serological properties of the virus isolates. The present paper is a report on some preliminary serological studies of viruses isolated from trees infected with members of the cherry-yellows group.

Materials and Methods

The isolates used in these experiments included: a strain of cherry yellows virus Y.4 causing a relatively mild symptom in Montmorency sour cherry; necrotic ring spot strain N.4 isolated from naturally infected peach, and strain N.5 inducing recurrent symptoms in sour cherry; two isolates of green ring mottle virus from sour cherry, G.1 and G.2; prune dwarf virus D.1 isolated from Italian prune and inducing mild shock but no yellows symptoms in sour cherry; and tatter leaf virus strains T.1 and T.2 from sweet cherry. The abbreviations designating virus strains are those used in other publications from this laboratory.

Virus entities associated with these diseases were transferred to cucumber from petals of Montmorency sour cherry trees or young leaves of peach trees in May, 1959. They were maintained in cucumber by successive transfers at intervals of from 10 to 14 days. The cucumber plants were propagated from seed and held in the greenhouse before and after inoculation. The experiments described below were conducted within 6 weeks of isolation from *Prunus* species long before the attenuation of the viruses generally accompanying repeated transfers to cucumber.

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Publication No. 13, Research Laboratory, Research Branch, Canada Department of Agriculture, Vineland Station, Ontario.

The virus extracts employed as antigens in serological tests were prepared from cucumber sources by differential centrifugation after clarification by dialysis of sap against a pH 4.6 buffer (method D (6)). Extracts of healthy cucumber leaf tissue were prepared by method D and also by method B (6) in which clarification was accomplished by the addition of sodium acetate buffer at pH 5.0 to sap extracted from frozen leaves. The supernatants obtained after the low-speed centrifugation of the first and second cycles of differential centrifugation are designated S_3 and S_5 respectively. Extracts prepared by the dialysis method are designated by the letter D prefixing the S_3 or S_5. Concentrations of preparations are indicated by the number of grams of leaf starting material per milliliter of solution. For example, N.5.DS_3 57× designates a preparation of N.5 purified from infected cucumber by the D method and each milliliter of the extract contains the yield of 57 grams of infected tissue.

To prepare antisera, 2½ ml of extract was emulsified with an equal volume of a mineral oil – Arlacel A mixture[2] (85:15 v/v). Emulsification into a solid cream was accomplished by stirring a mixture of the extract, mineral oil, and Arlacel A in the barrel of a 30-ml syringe for 7 minutes with a mechanical stirrer. The emulsion was then injected into the thighs of a rabbit. Four weeks later animals were bled to obtain antiserum. Three additional bleedings were made at weekly intervals. The antisera were lyophilized 6 months after collection.

The antisera are designated by the prefix "anti" followed by the code indicating the preparation used in the production of the serum. An extract of sour cherry petals prepared by a procedure described previously (5) was used in the production of anti-N.5.MP. Anti-Y.4.DS_5 and anti-H.DS_3 used in these experiments were obtained from the third and fourth bleedings respectively whereas anti-N.5.MP was composed of a pool of all bleedings.

Precipitin tests were carried out in 60-mm Petri dishes by the gel-diffusion technique at room temperature. Nine milliliters of 1% Difco agar in 0.6% sodium chloride solution at 50° C was poured into each Petri dish. The dishes previously had been coated with a formvar film to ensure a seal with the agar. Six peripheral wells 8 mm apart and 8 mm from a central well were made by placing seven pieces of glass tubing vertically in the liquid until the gel had solidified. One-tenth milliliter portions of antigen solutions and antisera were carefully pipetted into the wells with a syringe to avoid forcing liquid between the agar gel and the Petri dish. In titration experiments twofold serial dilutions of antigen or antiserum were placed in the outer wells around antiserum or antigen respectively in the center well. The distances between the antigen well and the precipitin zones were measured after 7 days. Measurements of sharp precipitin zones were accurate to 1 mm or better. The amount of precipitate in each zone was estimated independently by visual inspection by two observers and placed in one of four categories. Ratings assigned to each zone by the two observers agreed within one category of rating.

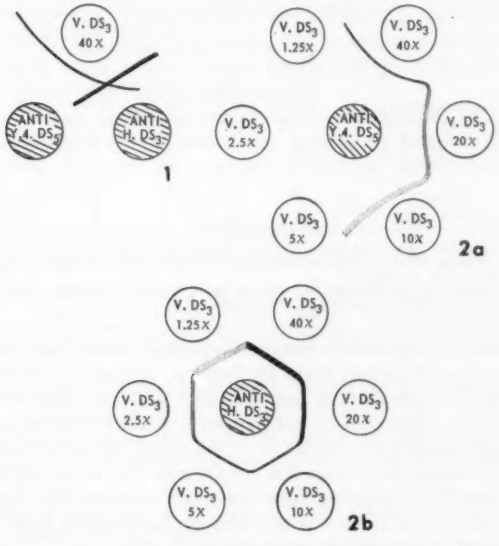
[2]Arlacel A (mannide monooleate) was supplied by the Atlas Powder Co. of Brantford, Ontario.

Results

The Precipitation of Virus and Host Antigens

Since it was necessary to know whether virus antisera reacted with host antigen(s), healthy cucumber material was prepared by four methods and tested with anti-Y.4.DS_5 and anti-N.5.MP. Extracts of healthy cucumber cotyledons and leaves prepared by the B and D methods (6), expressed sap, and sap clarified by low-speed centrifugation were employed. All tests with healthy cucumber material and virus antisera gave negative results. That the lack of precipitation was not due to either antigen or antiserum excess was demonstrated by titrating both antigens and antiserum. It should be emphasized that anti-N.5.MP was prepared from *Prunus* sources. In experiments at this laboratory, healthy *Prunus* petals and cucumber cotyledons have been found to have no demonstrable common antigen(s). It is reasonable to conclude that neither anti-Y.4.DS_5 nor anti-N.5.MP contained antibodies to cucumber antigens.

To determine the validity of the serological distinctions between virus and cucumber host antigens occurring in a mixture, a DS_3 40× preparation of infected cucumber was tested in the same plate with anti-Y.4.DS_5 and anti-H.DS_3. Crossing zones of precipitation, obtained between the virus preparation and both antisera (Fig. 1), demonstrated that the virus preparation contained two serologically distinct antigens, one of virus and one of host origin. Distances between the antigen well and the precipitin zones were 2 mm for the virus and 4 mm for the host antigen.



FIGS. 1 and 2. Precipitin patterns obtained by gel diffusion with antisera anti-Y.4. DS_5 and anti-H.DS_3. Antisera are represented by hatched and antigens by open circles.

The effects of dilution on the position and amount of precipitation were also investigated by titrating a D.1.DS_3 40× virus preparation in twofold serial dilutions against anti-Y.4.DS_5 and anti-H.DS_3. The virus antigen was precipitated 2 mm from the antigen well at the 40× concentration. As the virus preparation was diluted, the precipitation moved slightly closer to the antigen well (Fig. 2*a*). At the same time, the amount of precipitation gradually decreased. Similar observations were made with the host antigen (Fig. 2*b*) except that at the 40× concentration the precipitate was 4 mm from the antigen well. The differences in the position of precipitation resulted in a difference in precipitin pattern of virus and host antigens.

The Virus Antigens of Different Isolates

Preliminary experiments had shown that there was some degree of serological relationship between virus isolates of the cherry yellows group. Accordingly, DS_3 extracts of D.1, G.1, G.2, N.4, N.5, T.1, and T.2 were prepared simultaneously from cucumber sources to compare their reactions with anti-Y.4.DS_5, and anti-N.5.MP in a single experiment. In preparing the plates, both antigen extracts and antisera were carefully measured into the wells in 0.1-ml quantities with a tuberculin syringe. The antiserum was placed in the center well and the antigen extracts in peripheral wells. Three plates were used with each antiserum and the antigens were placed in a different order in each plate.

TABLE I

Reaction of two virus antisera with virus antigens in DS_3 preparations of seven isolates

		Antigen preparation						
Antiserum	Precipitate	D.1. DS_3 35×	G.1. DS_3 43×	G.2. DS_3 38×	N.4. DS_3 73×	N.5. DS_3 75×	T.1. DS_3 37×	T.2. DS_3 36×
Anti-Y.4.DS_5	Amount*	3	2	2	4	4	2	1
	Position†	2	1	1	3	3	1	1
Anti-N.5.MP	Amount*	1	4	3	4	4	1	4
	Position†	1	2	2	3	3	1	2

*The amount of precipitation was rated visually by two observers in four categories, 1 to 4. The figures given represent the arithmetic mean of the ratings of duplicates by two observers. Duplicated combinations did not vary by more than one rating of category.

†The position of precipitate is the distance between the precipitate and the edge of the antigen well to the nearest mm. Duplicates had identical position in every case.

When the plates were fully developed it was found that each zone had coalesced with the adjoining zone to form a precipitin pattern similar to that in Fig. 2*a*. The amount of precipitation and the distance between the precipitate and the edge of the antigen well varied with the antiserum as well as with the virus extract (Table I). For a given virus antigen, the amount and distance of precipitation was not necessarily the same for both antisera. For example, the T.2 virus antigen was precipitated by anti-Y.4.DS_5 in trace amounts at 1 mm from the antigen well, whereas with anti-N.5.MP it was precipitated in maximum amounts at 2 mm.

Two other examples of large differences in the amounts and distances of

precipitation with the two antisera were the G.1 and D.1 virus antigens. In contrast with the G.1 and T.2 virus antigens, the D.1 virus antigen reacted more strongly with anti-Y.4.DS_5 than with anti-N.5.MP. Since the concentrations of N.4.DS_3 and N.5.DS_3 were approximately double those of the other virus extracts, the amount of virus antigen apparently was too great to permit the detection of possible differences in reaction with the two antisera.

Discussion

These experiments showed that host and virus antigens in the same preparation could be readily distinguished by serological means: the absence of antibodies for host antigens in the virus antisera and vice versa enabled the two types of antigens to be readily identified; and the relative positions and the differences in pattern of the precipitin zones observed in the titration provide other criteria.

Antigens from different sources are generally considered to be related when they form coalescing precipitin zones with a single antiserum. Feinberg, however, stressed that this phenomenon should not be interpreted as signifying identity of antigens (1). In the present instance, several virus isolates from the cherry-yellows group have been shown to have common antigenic properties and, therefore, are serologically related. However, the differences in behavior of D.1, G.1, and T.2 with the Y.4 and N.5 antisera indicate that they are by no means identical. Apparently, the D.1 virus antigen has more antigenic groups with corresponding antibodies in anti-Y.4.DS_5 than in anti-N.5.MP, whereas the opposite is true of G.1 and T.2. Certainly, these results cannot be interpreted as meaning that one antiserum was more concentrated than the other. Conclusions regarding the smaller differences observed in precipitation with G.2 and T.1 were not made pending the completion of more extensive and critical experiments.

The viruses, whose antigenic properties were studied in this report, are not necessarily considered to cause the disease in *Prunus*. Indeed two distinct viruses, the tobacco and cucumber factors, have been found in G.1 and T.2 (2).

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THE PHYSIOLOGY OF HOST-PARASITE RELATIONS

IX. FURTHER OBSERVATIONS ON THE ACCUMULATION OF RADIOACTIVE SUBSTANCES AT RUST INFECTIONS[1]

MICHAEL SHAW

Abstract

Wang (Can. J. Botany, **38**, 635–642 (1960)) concluded that the accumulation of radioactivity observed on radioautographs at infection sites on rusted leaves fed with C^{14}-labelled substances was 'apparent' rather than real. The 'accumulation ratio' is defined as the ratio of the specific activities (c.p.m./mg dry weight of intact tissue) of rust-infected to uninfected areas of infected leaves. Theoretical considerations relating to the radioautography of leaves labelled with C^{14} and to the measurement of 'accumulation ratios' by extraction of C^{14}-labelled substances from rusted and uninfected segments of infected leaves, as well as experimental data, show that Wang's conclusion is not generally applicable.

Experimentally, it was shown using polymethacrylate C^{14} sources that differences in distance between sources and X-ray film of the order of 100 μ had no effect on the intensity of autoradiographs. Rust-infected leaves, fed with radioactive glucose, were radiographed between X-ray plates. Localization of radioactivity at infection sites was observed on both 'dorsal' and 'ventral' radiographs, indicating a real accumulation per unit area. Ventral were more radioactive than dorsal surfaces. The main development of the fungus occurred on the former. Radioautography revealed that C^{14} from glucose-1-C^{14}, glucose-6-C^{14}, and uniformly labelled glucose fed to excised wheat leaves became localized at 10-day-old rust infections in 2 hours. 'Accumulation ratios' calculated from the specific activity of leaf segments remained close to 1.0 for at least 6 hours after introduction of the tracer, but increased to more than 2 after 24 hours. When 'accumulation ratios' were calculated from the specific activities of individual pustules (excised with a punch 1 mm in diameter) and interpustular disks, values greater than 1 were observed in 2 hours, thus confirming the results of autoradiography. Differences between the 'accumulation ratios' observed with glucose-6-C^{14} and glucose-1-C^{14} were consistent with an increased role of the pentose phosphate pathway at infection sites. Incorporation of C^{14} from uniformly labelled glucose into the alcohol-insoluble fraction of rusted leaf segments was 2.5-fold that in uninfected segments in 6 hours and 3.65-fold in 24 hours. The humin formed during hydrochloric acid hydrolysis accounted for approximately 50% of the activity of the alcohol-insoluble material. The 'accumulation ratio' for the alcohol-soluble material was only 1.56 after 24 hours.

All the results support the view (Shaw and Samborski, Can. J. Botany, **34**, 389–405 (1956)) that there is a quantitative, metabolically dependent accumulation of C^{14} from radioactive glucose at vigorous rust infections. The relative roles of fungus and host in this process are discussed briefly.

Introduction

The uptake of radioactive compounds by leaves infected with rust or mildew has been investigated by Shaw and co-workers (1954 and 1956), by Yarwood and Jacobsen (1955), and more recently by Wang (1960).

The main conclusions reached by Shaw and Samborski (1956), and more particularly the basis upon which they were reached, do not always appear to have been clearly grasped by Wang (1960). Accordingly, they are set forth here in summary form:

(1) Radioautography showed that C^{14} sugars, etc., and P^{32} or radioactive

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Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask.

substances derived from them in the course of metabolism, became localized at infection sites when the tracers were fed to excised or attached leaves. The term 'infection site' was not precisely defined by Shaw and Samborski (1956). As used here it refers to both the fungus and the host tissue closely associated with it.

(2) The intensity of localization of radioactivity roughly paralleled the development of the parasite and the drift in respiratory intensity of leaf disks bearing infections.

(3) Although rust infection caused a local accumulation of dry matter per cm^2 of up to twofold at infection sites, when corrections for dry weight were made, the radioactivity from glucose-1-C^{14} accumulating at rust infections in 4.5 hours (leaf pieces floated on a tracer solution) and 12 hours (excised leaves with bases in tracer solution) was found to be approximately twofold that in noninfected areas of primary leaves of Little Club wheat; i.e., the activity per unit area at infection sites (disks 2.8 mm in diam.) was approximately fourfold that in nonrusted areas of the same leaf. No inferences were drawn from these observations concerning the histochemical distribution of radioactivity in the mycelium of the fungus or the host tissue at infection sites.

(4) Accumulation at rust infection sites of radioactivity from glucose-1-C^{14} was inhibited by anaerobiosis, by 'uncoupling' concentrations of 2,4-dinitrophenol (DNP), and by azide. The similar accumulation of activity at mildew infection sites was inhibited by DNP and by treatment with sulphur dust, which, it was specifically pointed out, kills the fungus. The effect of sulphur on accumulation at rust infections was not investigated. Since these treatments inhibit the respiration of both components of the host–parasite complex (see, for example, Table VII in Shaw and Samborski 1956), it was concluded only that accumulation at infections was "linked with their enhanced aerobic respiratory metabolism" (p. 402). In this sense, Shaw, Brown, and Jones (1954) had suggested earlier that the transport of substances to infection sites was an 'active' process.

(5) From the observation that radioactivity became localized at mildew infections on the day following the application of sulphur, when the fungus was assumed to be already dead, as well as from experiments with mildewed leaves from which the ectoparasitic mycelium had been removed, it was concluded that radioactivity accumulated in the host tissue at the infection sites of this fungus. It was also noted that when glucose-1-C^{14} was fed to mildewed leaves 3 days after the application of sulphur, there was no localization of activity at infection sites. This illustrates the essential role of fungal metabolism in the process of continued accumulation.

(6) Shaw and Samborski (1956) also pointed out specifically that the fungal mycelium and spores of both rust and mildew became radioactive when infected leaves were fed with tracers. With reference to microradioautographs of microtome sections of rust-infected leaves previously fed with glucose-1-C^{14}, they commented on the diffusion error of their technique and stated: "they did suggest, however, that the bulk of the radioactivity at infections was in the host tissue and provided a clear demonstration of activity in

developing uredospores and teliospores. Further work on the histological distribution of activity at the infections is necessary" (p. 400).

(7) In discussing their results they stated further: "A simple view would be that the substances accumulate directly in the fungal mycelium and developing spores" (p. 402). They then cited indirect evidence to suggest that stimulation of the metabolism of host tissue plays a role in the process of accumulation at rust infections and concluded that the evidence supported the view that "the various substances accumulate in the tissues of the host, whence they may become available to the parasite" (p. 402). They expressed the view that "the rapid transport of radioactive substances toward and their accumulation at infections is probably similar to the accumulation of radioactive substances in meristematic or other rapidly metabolizing regions" (p. 404).

(8) They reported an enhanced fixation of carbon dioxide at the periphery of infection sites during short (ca. 4 minutes) exposures to radioactive carbon dioxide in the light. An enhanced dark-fixation of carbon dioxide at infection sites is suggested by their observations (e.g., their Fig. 31) although they did not comment on this possibility. No quantitative data are available for the rates of carbon dioxide fixation at infections, either in the light or in darkness.

Translocation toward and carbon dioxide fixation at infection sites supply the parasite with the substances necessary for its growth. The relative importance of the two processes in supplying carbon skeletons may be assumed to vary with the environmental or experimental conditions; mineral elements are clearly supplied by transport.

It will be apparent from the foregoing that although Shaw and Samborski (1956) stressed the role of the host tissue at infection sites, they were well aware of the critical role played by the parasite in the process of "accumulation".

Wang (1960) observed a moderate (see his Fig. 1) accumulation of radioactivity from uniformly labelled glucose on radiographs of rust-infected Little Club leaves. When he measured the radioactivity in the alcohol-soluble material and in the supernatant from the hydrochloric acid hydrolyzate of the alcohol-insoluble material, he found less radioactivity in the rusted than in the healthy areas of infected leaves. No mention was made of the measurement of the activity of the humin formed during hydrolysis, so that it is possible that Wang's quantitative estimates do not account for all the radioactivity in the tissue. Wang (1960) concluded that an 'apparent' accumulation is observed on radiographs because radioactivity accumulates in the actively metabolizing mycelium and spores and because the mycelium and spores develop on one side of the infected leaf (the 'pustule' side) and thus come more closely into contact with the X-ray film. It is important to notice that accumulation of radioactivity in the fungus because of its high metabolic rate falls within the meaning of the term 'accumulation' as used in the present paper and by Shaw and Samborski (1956). Wang (1960) did not, however, concede that a quantitative accumulation of activity per unit weight of tissue (fungus plus host) could occur at infection sites and made no reference to the published data (see Tables IV and V of Shaw and Samborski 1956, and Yarwood and Jacobson 1955) which illustrate this point.

A theoretical discussion of problems involved in the gross radioautography and quantitative measurement of radioactivity in infected leaves, together with illustrative experimental data, is presented below.

Theoretical

A. Radioautography

Three factors related to the structure of uninfected and parasitized leaves affect the results obtained by radioautography. These are: (1) the thickness and density of the tissue, (2) the distribution of activity within the leaf, and (3) the closeness of contact with the X-ray film. Each of these will be discussed in turn.

Thickness and density.—Dried, uninfected, primary wheat leaves are about 90 ± 5 μ thick and weigh 2.2 ± 0.2 mg/cm^2. Measured at a pustule of *Puccinia graminis* about 10 days after inoculation, thickness increases to about 160 ± 5 μ and the weight may be doubled. Beta rays from C^{14} have an average energy of about 0.05 Mev and a maximum energy of about 0.16 Mev (Calvin *et al.* 1949). Self-absorption (as used here the term includes some effect of scattering) in finely ground wheat leaf tissue is still incomplete at a thickness corresponding to 22 mg/cm^2, which is equivalent to a layer approximately 10 leaves thick. Thus even assuming a doubling of the dry weight per cm^2 at infection centers and a uniform concentration and distribution of activity throughout infected and uninfected areas, it follows that radioautography will result in an increased intensity of fogging of the X-ray film at infection sites. In fact, since self-absorption amounts to only approximately 20% at 2 mg/cm^2 and to approximately 40% at 4 mg/cm^2, an increase in the intensity of fogging could occur even if the concentration of the isotope at infections were appreciably below that in uninfected tissue. This was recognized by Shaw and Samborski (1956) as the most important source of error in the autoradiography of leaves.

Nonuniform distribution.—If, without any change in the total radioactivity per unit area, radioactivity is concentrated at one surface of the leaf, self-absorption effects at this surface will be lower than at the other. The distribution of radioactivity at infection sites is certainly not uniform because the fungus becomes radioactive. *Erysiphe* grows on the leaf surface; *Puccinia* tends to develop mainly beneath the ventral (abaxial) surface of the wheat leaf. It will, however, be clear that the thickness and density of the tissue at infection sites and elsewhere are too small for the nonuniform distribution of radioactivity to be a major source of error.

Contact.—Poor contact results in poor resolution because of increased scatter of the β rays. If for the moment a tissue of uniform density and also a uniform distribution of radioactivity are assumed, locally elevated areas such as rust pustules and veins might be expected to cause somewhat greater fogging of the X-ray film because they are in closer contact with it. The range of the β particles in air is very great (many centimeters) compared with their range in the tissue (50% of C^{14} β particles have a range less than 27 μ in tissue with a density of 1 g/cm^3). The density of the tissue both at infections and elsewhere is of the order of 0.25 g/cm^3 and the differences in

thickness (or elevation) between infection sites and surrounding tissue are only of the order of 100 μ. Thus the effect of differences in contact in suggesting an accumulation of activity where none exists is of a minor order of magnitude.

B. Quantitative Measurement of Radioactivity

Estimates of localized accumulation, based on measurements of total radioactivity per unit weight of an area of tissue within which the sites of accumulation lie, may yield results that are much too low because of the 'dilution effect' of the tissue between the sites of accumulation.

This may be illustrated as follows: Fig. 1 is a diagram of a wheat leaf having an infected area (I) lying between uninfected areas (H). A mean pustular area of 1 mm² and a pustular frequency of 12 per cm² are assumed. (These figures correspond almost exactly to those given for a pustular frequency of 20 per 3 cm length of leaf in Table III of Samborski and Shaw (1956).) The area of the interpustular tissue in I is therefore 0.88 cm². The dry weight of

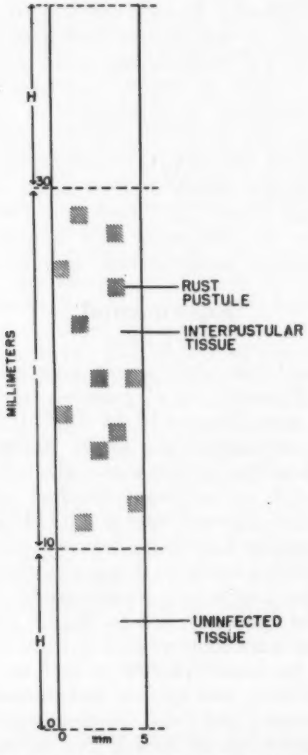


FIG. 1. Diagram of rust-infected wheat leaf. For explanation, see text.

the interpustular tissue in I may be taken as 2.2 mg/cm² for a total of 1.94 mg. The dry weight of the pustules (or infection sites) is taken as 2×2.2 mg/cm² for a total of 0.53 mg. The total dry weight of I is therefore 2.47 mg.

It is assumed that after feeding with tracer, the interpustular tissue has unit activity (1.0) per mg dry weight (i.e. a total activity of 1.94) and that the pustules have double this activity (i.e. a total of $0.53 \times 2 = 1.06$) so that the 'accumulation ratio' at infections is 2.0. The total activity for I thus becomes $1.94+1.06 = 3.0$. But the activity per mg is only $(1.94+1.06)/2.47$, that is 1.21. If the dry weight and activity of H are taken as equal to those for the interpustular areas in I, the quantitative determination of the relative activities in I and H will now yield an 'accumulation ratio' of only 1.21, a figure that is too low by 80% (case A).

In this model all the accumulated radioactivity at the pustules could be accounted for by a depletion of the radioactivity of the interpustular areas amounting to 27% ($0.53 \times 100/1.94$). That is, after feeding with tracer the specific activity of H areas is still taken as 1.0 and that of the pustules as 2.0, but that of the interpustular areas is now only 0.73. If this occurred, the quantitative determination of the relative activities in I and H would yield an 'accumulation ratio' of 1.0, representing no accumulation at all! (Case B.)

On the other hand, in both A and B, radioautography would correctly show the existence of a marked 'accumulation' of activity at the infection sites. Assuming that the ratio of activities in interpustular and H areas does not in fact fall below say 0.5, the importance of the 'dilution effect' in masking measurement of accumulation clearly declines as the ratio of the activities in pustules and H areas rises above 2.0. Clearly also the separation of pustules from interpustular areas is a necessary and important step in the exact quantitative measurement of 'accumulation' at infection sites.

Experimental

METHODS

Dwarf sunflowers and Little Club wheat were grown in the greenhouse and inoculated with *P. helianthi* and *P. graminis tritici* (Race 15B), respectively. The leaves were ready for use 10–12 days after inoculation, at which time the rust pustules were sporulating freely. Excised leaves were exposed to $C^{14}O_2$ in the light as described by Shaw and Maclachlan (1954), or fed with glucose-1-C^{14}, glucose-6-C^{14}, or uniformly labelled glucose as described by Shaw and Samborski (1956). One milliliter of a 0.114% solution with an activity of 1.0 or 0.1 μc was used per 12 primary leaves of wheat. The tracer solution was replenished with distilled water as it was absorbed. During 'feeding' the vials holding the leaves were kept on a greenhouse bench in the shade. Leaves were 'harvested' from the vials at 2, 4, 6 (or 8), 12, and 24 hours after introduction of the tracer and were then washed quickly in running tap water.

For radioautography, the basal 'quarter' of each leaf, which had been submerged in the tracer solution, was excised and discarded. The remainder of the leaf was carefully pressed and dried. Radioautography of leaves and C^{14} polymer sources was carried out on Kodak (No Screen) medical X-ray film, employing exposure periods of up to 1 week. In order to obtain radiographs

of both dorsal and ventral surfaces, some leaves were radiographed between two X-ray films. Care was then taken to develop the two films simultaneously under identical conditions. Quantitative measurements of the intensity of fogging of the film were made with a microphotometer similar to that described by Swift and Rasch (1956). The transmission of unexposed portions of the radiograph was taken as 100%. Illustrations of radiographs given in the paper are prints and areas of high activity are therefore white.

For direct quantitative estimation of radioactivity, the leaves were cut into 4 'quarters' numbered from the base to the tip. For these experiments wheat leaves were inoculated so as to produce a 'rusted' section approximately 2 cm long, the remainder of the leaf being free of infection. The 'quarters' were not uniform in length, but were cut so that the third quarters contained all the rust pustules (see Fig. 1). Corresponding quarters from 20 leaves were pooled, dried overnight in the oven at 95° C, and finely ground in a small mortar. Samples were weighed into stainless steel dishes, spread as uniformly as possible, and counted in a CFIS gas flow counter (Atomic Energy of Canada) to a standard error of 2%, using a constant geometry. Counts were corrected for background and, from an empirically constructed curve, for self-absorption. The counts were not large enough for coincidence corrections to be necessary. 'Accumulation ratios' (Shaw and Samborski 1956) were calculated by dividing the specific activity (c.p.m./mg dry weight) of rust-infected quarters by the mean specific activity of the second and fourth quarters. In other experiments individual rust pustules were excised either from pressed, dried leaves with a fine scalpel or from fresh leaves with a punch 1 mm in diameter (see Fig. 21). Samples of uninfected tissue were taken in like manner from between the pustules. Rusted and uninfected samples were then dried and counted as described above. 'Accumulation ratios' were then calculated by dividing the specific activity of the pustules by that of the interpustular tissue. Excision of leaf disks from fresh leaves eliminates any error due to movement of tracer during drying.

In a brief examination of the distribution of radioactivity from uniformly labelled glucose between soluble and insoluble matter, the following procedure was employed. Dried and ground leaf material was refluxed for two half-hour periods in boiling 80% alcohol. The alcohol was removed *in vacuo* at 40° C and the aqueous residue brought to 1 ml. The alcohol-insoluble material was recovered, dried, weighed, and then hydrolyzed in 6*N* HCl in sealed pyrex tubes at 120° C for 24 hours (Shaw and Colotelo 1961). The humin was recovered, washed four times, dried, and weighed. The supernatant was freed of HCl by evaporation to dryness and brought to a volume of 1 ml. Aliquots (50 μl) of the alcohol-soluble and of the hydrolyzate of the alcohol-insoluble material and the complete humin fraction were counted in stainless steel dishes as described above.

RESULTS

A. Autoradiography

C^{14} Polymer Sources

A sheet of C^{14} polymethacrylate polymer (Nuclear Chicago CFP2, 3×3× 0.18 cm) was placed on an X-ray film. One edge of the polymer was separated

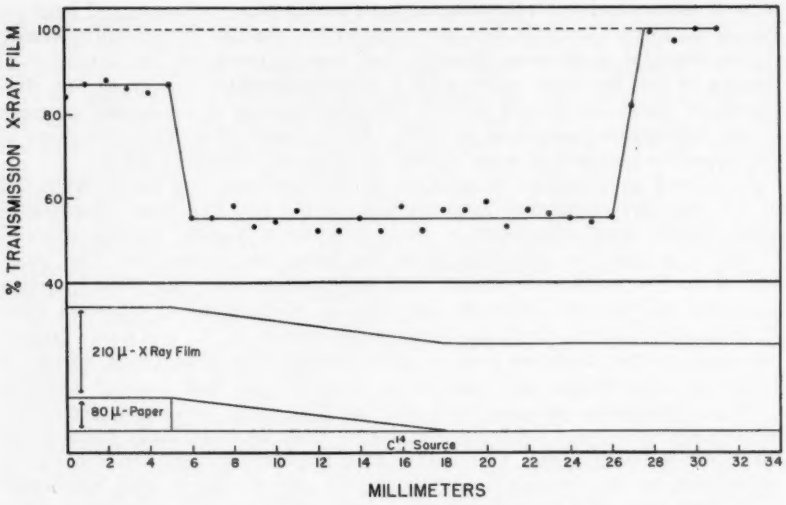


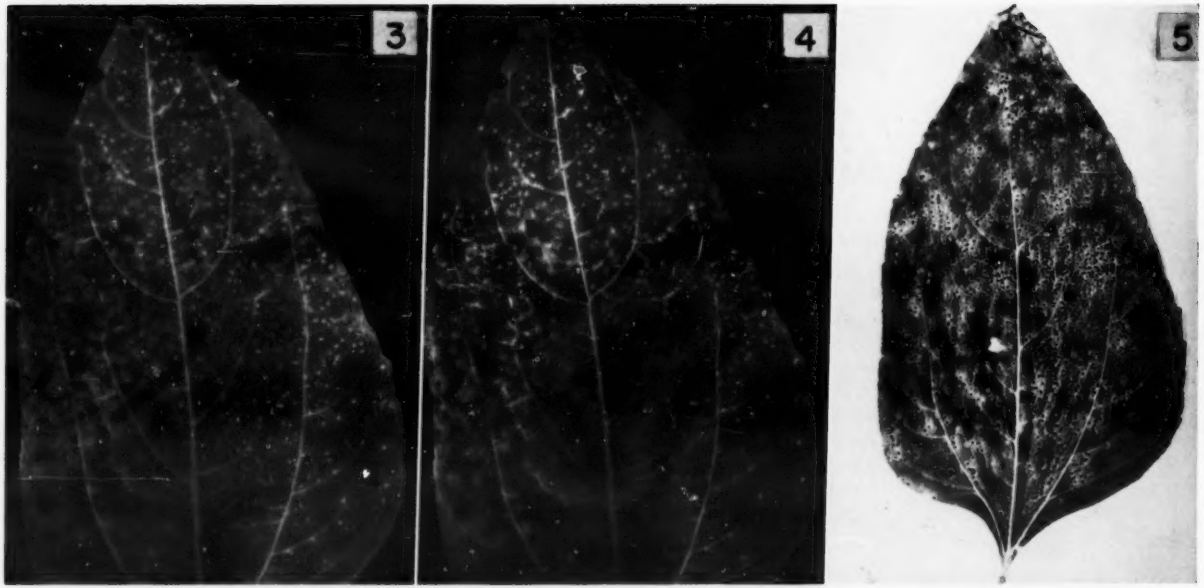
FIG. 2. Bottom: diagram of arrangement of polymethacrylate C^{14} source, bond paper, and X-ray film.
Top: transmission curve of radioautograph obtained using arrangement shown in bottom half of figure.

from the film by a sheet of bond writing paper 80 μ thick. The whole was then sandwiched between thick, flat glass plates. With this arrangement the distance between the surface of the film and the surface of the C^{14} polymer varied from 80 μ at the edge of the bond paper to zero (Fig. 2).

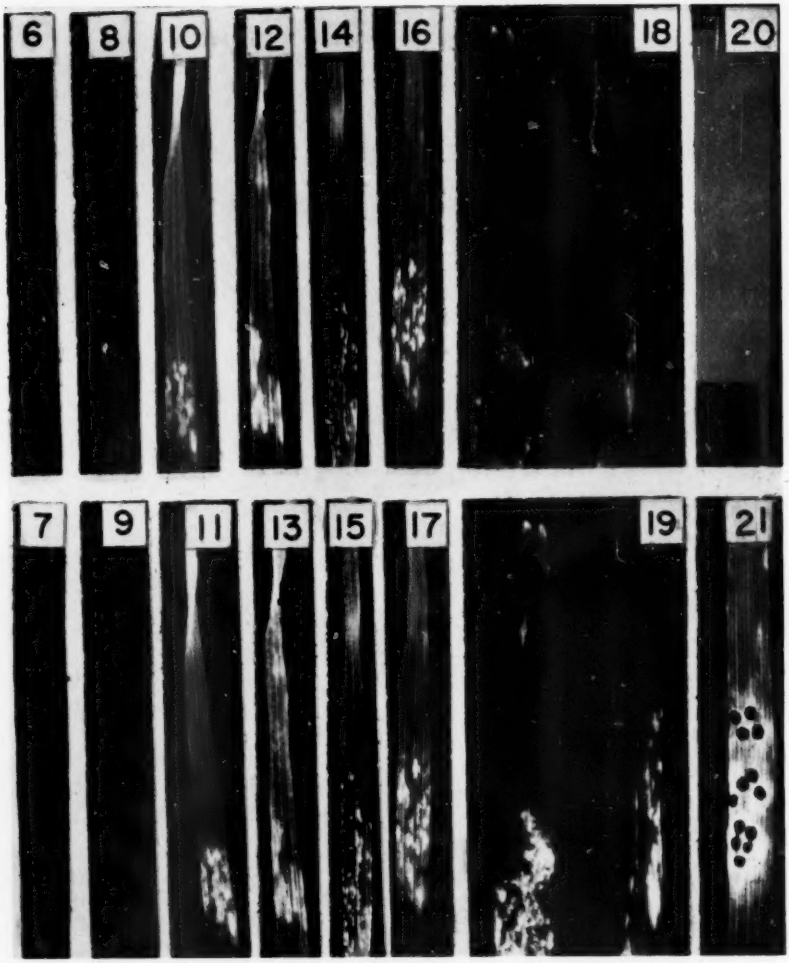
There was no visible effect of the difference in distance between the C^{14} source and the film on the intensity of fogging in the original radioautograph or in the print in Fig. 7, or in the transmission measurements in Fig. 2. The latter shows no evidence of a decline in transmission with increasing distance from the edge of the bond paper. The effect of the paper is clearly visible in Figs. 2 and 20. Transmission of the exposed X-ray film never fell below 40% of that of unexposed film.

In a variant of this experiment two circular flat-bottomed holes (approximately 130 μ and 80 μ deep) were machined into a CFPI C^{14} polymer source. This was then radiographed with the holes facing the X-ray film. There was no reduction in the intensity of fogging of the film at positions corresponding to the holes on the resulting radiograph.

These results are predictable from theory and show that differences in distance between C^{14} source and X-ray film of the order of 100 μ make no visible difference to the intensity of blackening of the film under the usual conditions employed in the autoradiography of leaves. Differences of this order do affect the degree of resolution obtained on the radiographs and this, too, can be simply and easily demonstrated.



FIGS. 3, 4, and 5. Prints of dorsal and ventral radioautographs and photograph of ventral surface of rust-infested sunflower leaf after 20 minutes' exposure to $C^{14}O_2$ in the light.



FIGS. 6–17. Prints of dorsal (Figs. 6, 8, 12, 14, and 16) and ventral (Figs. 7, 9, 11, 13, 15, and 17) radioautographs of rust-infected wheat leaves fed with glucose-1-C^{14} (Figs. 6, 7, 10, 11, 14, and 15) or glucose-6-C^{14} (Figs. 8, 9, 12, 13, 16, and 17) for 2 hours (Figs. 6–9), 4 hours (Figs. 10–13), and 6 hours (Figs. 14–17).

FIGS. 18 and 19. Prints of dorsal (Fig. 18) and ventral (Fig. 19) radioautographs of rust-infected wheat leaves fed with uniformly labelled glucose for 24 hours.

FIG. 20. Print of radioautograph of polymethacrylate C^{14} source separated at one end (see bottom of figure) from an X-ray film by a piece of bond paper.

FIG. 21. Print of radioautograph of radioactive rust-infected wheat leaf after excision of infection centers with 1-mm leaf punch.

Leaves

Rust-infected sunflower leaves were exposed to $C^{14}O_2$ (20 μc in approximately 20 l. of air; total CO_2 concentration 0.04% v/v) at a light intensity of about 800 ft-c for 20 minutes. Figures 3, 4, and 5 illustrate respectively simultaneous radiographs of the dorsal and ventral surfaces and a photograph of the ventral surface of a single leaf. Uredia developed mainly on the ventral surface. Photosynthetic fixation of $C^{14}O_2$ occurs only in the host tissues. No gross differences in the distribution of radioactivity at infection sites or in the intensity of the two radiographs were observed.

Figures 6 to 17 illustrate simultaneous radiographs of dorsal and ventral surfaces of rust-infected wheat leaves after feeding with either glucose-1-C^{14} or glucose-6-C^{14} for 2, 4, and 6 hours. Examination of the original radiographs showed more clearly than the prints in Figs. 6 to 17 that the intensity of localization of radioactivity was greater on the ventral than on the dorsal surface. The uredia developed mainly in the ventral surface. An interesting point is that the difference in intensity of localization between ventral and dorsal radiographs increased with the passage of time after introduction of the tracer. This effect is evident in Figs. 6 to 17 but is particularly well illustrated by Figs. 18 and 19, which are prints of dorsal (Fig. 18) and ventral (Fig. 19) radiographs of leaves obtained after feeding with uniformly labelled glucose for 24 hours. This result indicates the progressive accumulation of

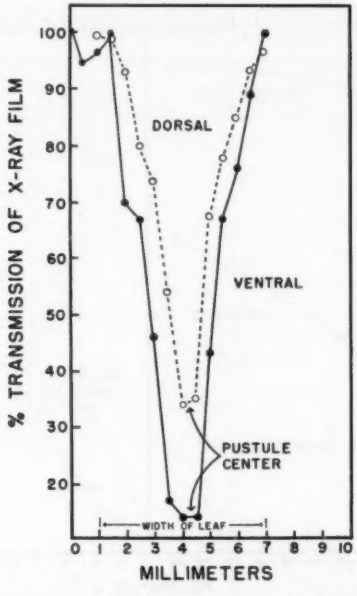


FIG. 22. Transmission curves of dorsal and ventral radiographs of a single radioactive rust pustule.

radioactivity in the fungus (uredospores and mycelium) or the host cells closely associated with it or both. The host cells are specially mentioned because there is no basis for excluding them at this juncture. It is not to be implied that the radioactivity is uniformly distributed in the host cells at infection sites.

The difference in intensity between ventral and dorsal radiographs is expressed quantitatively by the transmission curves for a single pustule presented in Fig. 22.

The differences between ventral and dorsal radiographs described here represent the integrated effect of the factors described under Theoretical, part A. They are due mainly to differences in the degree of self-absorption at the two surfaces and indicate that the radioactivity is not homogeneously distributed throughout the thickness of the tissue at infection sites, but is higher on the ventral surface on which the main development of the fungus occurs. They indicate that radioactivity accumulates in the fungus itself but do not preclude accumulation in the host cells closely associated with the fungus. They also show beyond dispute that there is a real and not merely an 'apparent' accumulation of radioactivity per unit area at infection sites.

B. Quantitative Measurements of 'Accumulation' at Infection Sites

Figure 23 illustrates the drifts with time in the mean accumulation ratios for glucose-1-C^{14} and glucose-6-C^{14} and in the ratios for uniformly labelled

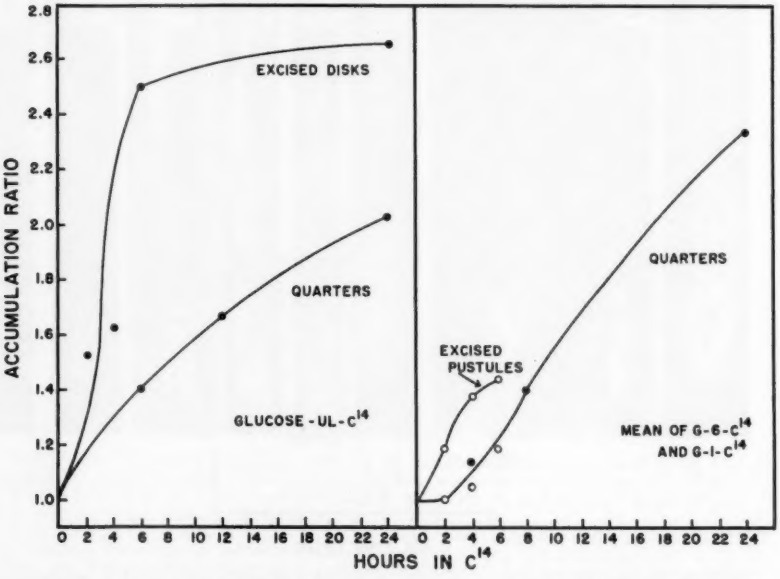


FIG. 23. 'Accumulation ratios' for leaf quarters and excised infections vs. time in C^{14} solution.

glucose obtained by the leaf quartering technique (see p. 1399). Using this technique a quantitative accumulation of radioactivity in rusted (third) quarters was not detected until at least 6 hours after the introduction of the tracer. On the other hand, radiographs of individual leaves from the same experimental runs showed an unmistakable localization of activity at infection sites after 2 hours (see Figs. 6 to 9). It will be clear that 'accumulation ratios' calculated from data obtained in this way are subject to the 'dilution error' discussed on pages 1397–98. In addition, in the earlier stages of feeding at least, there is clearly a gradient of radioactivity from the base to the tip of the leaf, so that the specific activity of the tip (fourth) quarter is less than that of the second quarter. This is illustrated by the data in Table I. A similar gradient exists in uninfected leaves.

The dilution error is minimized when excised 'pustules' are compared with interpustular tissue and the effects of the linear gradient in activity are also largely avoided (see Fig. 21 and Methods). Results obtained by excision of pustules and interpustular tissue are also illustrated in Fig. 23. They show that, in agreement with the results of radioautography, there is an unmistakable quantitative accumulation of radioactivity at infection sites after only 2 hours under the conditions of these experiments. It must be noted that the 'accumulation ratios' refer to specific activities (c.p.m./dry weight).

TABLE I

Accumulation of radioactivity from glucose-6-C^{14} and glucose-1-C^{14} in rust-infected leaf quarters

Hours fed	Label	Leaf quarter	C.p.m./mg dry weight	Accumulation ratio
4	C_6	1	818	
		2	688	1.3
		3R	658	
		4	329	
4	C_1	1	890	
		2	878	0.97
		3R	637	
		4	430	
6	C_6	1	586	
		2	531	1.48
		3R	717	
		4	435	
6	C_1	1	481	
		2	494	1.31
		3R	570	
		4	375	
24	C_6	1	519	
		2	308	2.29
		3R	726	
		4	325	
24	C_1	1	629	
		2	262	2.38
		3R	574	
		4	219	

Using leaf disks 2.8 cm in diameter it was found earlier that the dry weight of disks bearing infections was double that of disks from uninfected leaves. Disks cut with the 1-mm punch had an average dry weight of 6.7 mg/cm² when centered on infections approximately 10 days old. The dry weight of interpustular disks was only 2.2 mg/cm². Calculated per cm², the 'accumulation ratios' for excised disks shown in Fig. 23 would thus be increased by a factor of three (6.7/2.2) over the values indicated.

C. Comparison of Glucose-1-C¹⁴ and Glucose-6-C¹⁴

The tracers were fed in the usual manner, except that after about 6 hours, when all the original solution had been absorbed, the leaves were washed quickly in running water and floated on distilled water in Petri dishes. Samples were harvested at 4, 8, and 12 hours. The detailed results are given in Table I. When harvested, the leaves were again washed briefly in running water, quartered, and dried in the oven.

In the early stages (4 hours) there was a gradient of activity from the first to the fourth quarter. Whether or not the presence of rust infections impedes the transport of radioactive glucose into the fourth quarter (see Wang 1960) is difficult to say. However, a gradient of activity occurs in uninfected leaves and when tracers are fed to leaves with infections in the second, third, and fourth quarters, radioactivity accumulates at all infection sites. By 24 hours it was evident that the two metabolically more active quarters, namely the first or basal quarter containing the younger tissue and the rust-infected third quarter, had accumulated the greatest amounts of radioactivity. The data strongly suggest that quarters 2 and 4 lost activity to quarters 1 and 3. In this connection it should be noted that the increase in respiration rate caused by rust infection is sharply localized at infection sites (Samborski and Shaw 1956). Loss of radioactivity as carbon dioxide is thus most rapid at infection sites where accumulation of radioactivity is greatest. The error due to photosynthetic refixation of respiratory $C^{14}O_2$ is negligible (Shaw and Samborski 1956).

Rust infection lowers the C_6/C_1 ratio because the No. 1 carbon of glucose is rapidly converted to carbon dioxide in the pentose phosphate cycle (Shaw and Samborski 1957). If glucose-6-C¹⁴ and glucose-1-C¹⁴ are fed continuously to infected or uninfected tissue the C_6/C_1 ratio increases with time because of recycling. Accordingly, it would be predicted that the 'accumulation ratio' observed with glucose-6-C¹⁴ should be at first higher than that observed with glucose-1-C¹⁴, that the 'accumulation ratios' observed with glucose-1-C¹⁴ should increase as the length of the feeding period is increased, and that they should ultimately be the same as those observed with glucose-6-C¹⁴. Within the limits of experimental error these predictions are fulfilled by the data in Table I.

D. Distribution of Radioactivity (from Uniformly Labelled Glucose) between Alcohol Soluble and Insoluble Matter

Uniformly labelled glucose was fed to rust-infected primary leaves of Little Club wheat for 6, 12, and 24 hours. At each 'harvest' the leaves were quartered and the corresponding quarters were pooled, dried, ground, and counted in the usual manner. The second and fourth quarters were then mixed

TABLE II

Distribution of radioactivity from uniformly labelled glucose in the alcohol soluble and insoluble material in rust-infected and uninfected leaf quarters

Hours fed	Dry weights (total = 80 mg)				Total activity (c.p.m./fraction)				
	Alcohol insoluble			Alc. sol.	Alcohol insoluble			Alc. sol.	Sol. + insol.
	Supernatant	Humin	Sup. + hum.		Supernatant	Humin	Sup. + hum.		
6H	40.4	8.5	48.9	31.1	7,040	6,945	13,985	29,160	43,145
6R	51.4	9.0	60.4	19.6	19,920	15,219	35,139	32,360	67,499
6R/H							2.51	1.11	1.56
12H	35.3	9.8	45.1	34.9	13,140	15,239	28,379	37,760	66,139
12R	43.0	9.8	52.8	27.2	38,680	40,366	79,046	53,140	132,186
12R/H							2.79	1.41	2.00
24H	34.9	8.7	43.6	36.4	10,760	17,061	27,821	41,060	68,881
24R	42.2	9.5	51.7	28.3	47,900	53,514	101,414	63,980	165,394
24R/H							3.65	1.56	2.40

to provide a single 'healthy' sample. Subsamples (80 mg dry weight) of healthy and rusted (third quarter) samples were then extracted with alcohol and hydrolyzed as described on page 1399 to yield alcohol soluble and insoluble (supernatant and humin) fractions. The dry weights and relative total activities of these fractions are listed in Table II. The specific activity of the healthy sample was less than the mean specific activity of the second and fourth quarters. Accumulation ratios calculated from the total activities of the various fractions are accordingly approximately 20% higher than those calculated from the specific activities of unextracted quarters.

The data in Table II show that the difference between the activity of healthy and rusted quarters lies chiefly in the greater activity of the alcohol-insoluble fraction of the latter. Thus the accumulation ratio for the alcohol-soluble material rose only to 1.56 in 24 hours, whereas that for the alcohol-insoluble material rose to 3.65. This clearly indicates that the accumulation of radioactivity at rust infections is largely the result of metabolic incorporation of the glucose and that accumulation of the free glucose plays a relatively minor role. The activity of the humin formed on hydrolysis accounted for from about 49% (rusted) to about 55% (healthy) of the activity of the alcohol-insoluble material. Disregard of the humin, a fraction frequently consigned to the sink (see, for example, Wang 1960), can therefore lead to a 50% underestimate of the extent to which glucose is incorporated into insoluble material and to approximately an 8% underestimate of the over-all 'accumulation ratio'.

Conclusions

Both theory and experiment support the conclusion reached earlier (Shaw and Samborski 1956) that there is a quantitative, metabolically dependent, accumulation of radioactivity from radioactive glucose at rust infections and that this is correctly revealed by radioautography. The accumulation at rust infections noted by Wang (1960) on radiographs, though moderate, was undoubtedly real, at least on a unit area basis, rather than 'apparent' as he suggests. His failure to find a higher activity in extracts of rusted areas of wheat leaves is considered to be due to the cumulative action of: (1) his use of a relatively short feeding period (3.5 hours), (2) his disregard of the humin fraction, (3) the 'dilution effect' (see pp. 1397–98). In this connection it is pertinent that the 'dilution effect' operates within single pustules when they become senescent, since radioactive substances then no longer penetrate to their centers (Shaw and Samborski 1956). Much less accumulation occurs at infection sites after the respiratory maximum is passed (Shaw and Samborski 1956) when the fungus is no longer growing or sporulating vigorously. In one sense the primary cause of accumulation is clearly the metabolic activity of the fungus—accumulation occurs at infections and activity does accumulate in the fungus—but the participation of stimulated host cells in the over-all process cannot be eliminated at this juncture. The extent to which there is increased incorporation of radioactive substances and accumulation of radioactivity by such host cells remains unsettled. Microphotometric determinations of the RNA – azure B complex strongly suggest an increase in the RNA content of the nuclei of the host cells at rust infections (Whitney 1958).

Nonetheless, my opinion at present is that the role of the host cells at infection sites is quantitatively less important than Shaw and Samborski were prone to think in 1956. It seems likely that the host may contribute a much smaller fraction to the total increase in dry weight at rust infections on cereal leaves than these authors thought. Shaw and Colotelo (1961) have suggested that the host's contribution may not exceed 25% of the total gain in weight. To this extent I agree with Wang's (1960) emphasis on the role of the fungus.

Acknowledgments

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VARIABILITY AND HYPHAL ANASTOMOSES IN HOST-SPECIFIC FORMS OF MARSSONINA POPULI (LIB.) MAGN.[1]

M. G. BOYER[2]

Abstract

Cultural studies, including observations on anastomoses, were undertaken on host-specific forms of *Marssonina populi* (Lib.) Magn. The fungus appears to consist of a highly variable and unstable series of forms differing in both morphological and cultural characteristics. Cytological studies have indicated that the pathogen is a uninucleate fungus. Anastomoses, evident between most of the forms examined, frequently resulted in dicaryotization of one of the cells but the dicaryotic condition was not perpetuated.

Introduction

The genus *Marssonina* (12) consists of a large number of parasitic species that cause anthracnose of herbaceous and woody plants. On the foliage and shoots of a susceptible host the fungus causes small, discrete, circular or lens-shaped lesions, containing at the center a gelatinous mass of two-celled, ovate or pyriform conidia in subcuticular acervuli (Fig. 1).

Among woody plants, species and hybrids of the genus *Populus* L. are often severely attacked by *Marssonina*. In the province of Quebec, all the native species and many recently introduced hybrid poplars are susceptible (Figs. 2, 3). Several hybrids, notably *P. nigra* var. *betulifolia* × "*Volga*", *P. deltoides* × "*Volga*", and *P. charkowiensis* × "*robusta*", are frequently and severely attacked (3). In the case of *P. deltoides* × "*Volga*", under the environmental conditions existing in the province, the effects of the disease are evident in suppressed growth and an increased susceptibility to winter injury. At the present time only limited information is available on the importance of this disease on other hybrids, for although several have been observed prematurely defoliated, observations were recorded late in the growing season (September), and nothing is known of the time at which the disease attained severe proportions.

Several reports (9, 10, 14) indicate the possible influence that this disease exerts on the growth and vigor of poplar. In the author's experience few native poplars are seriously affected, but it appears that some introduced poplars are sufficiently susceptible to warrant a more thorough study of this ubiquitous fungus and its possible detrimental role in poplar culture.

In the past, several species of *Marssonina* parasitic on poplar have been proposed but Thompson (19), in attempting to clarify the taxonomy of the fungus, examined exsicattae of over 15 species in 32 collections and concluded that, while spore size and shape (the apparent criteria for separation) were highly variable, the group was taxonomically inseparable. Additional studies

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on the perfect stage, *Drepanopeziza populorum* (Desm.) v. Höhnel, of a limited number of species supported this interpretation. The perfect stage of the form generally known as *Marssonina castagnei* (Desm. and Mont.) Magn., which is parasitic on *Populus alba* and presumably also on *P. alba* var. *nivea*, was one of those examined. This specific name, in addition to *M. populi* (Lib.) Magn., *M. curvata* (Bob. and Kab.) Magn., *M. piriformis* (Riess) Sacc., *M. nigricans* (Ell. and Ev.) Magn., and *M. populi-nigrae* Kleb., is probably the most frequently found in the literature. In this paper all isolates were included under the name *M. populi* (18, 19) although they often showed distinctive but variable morphological differences. Evidence of host specificity among isolates of *M. populi* was demonstrated in North America by Thompson (19), who separated three forms of the organism isolated from *P. tremuloides*, *P. alba*, and *P. balsamifera* (*P. deltoides*) respectively. The isolate from *P. deltoides* was pathogenic on 16 species and hybrids of the *Aigeiros* and *Tacamahaca* groups (20), but was nonpathogenic on *P. alba* and *P. tremuloides*. The isolate from *P. alba* parasitized only that host and *P. epirotica* (*P. alba* var. *nivea*), while the form from *P. tremuloides* was limited to that species.

Hyphal anastomoses between forms of *M. populi* have not been reported previously in the literature. Since it was a commonly observed phenomenon among most isolates reported in this study and its importance in the formation of new forms or races has been well established in other fungi (6, 8, 11), the process was examined in some detail.

As early as 1914, Burgeff (5) suggested that anastomoses between two strains might result in hybridization. Since that time the vegetative induction of new strains varying in pathogenicity or morphological characteristics has been repeatedly attained in some (1, 13, 16), but not all (7), heterocaryotic fungi. In *M. populi* both the mycelial and spore cells of all isolates studied were uninucleate or very rarely bi- or tri-nucleate; therefore the organism cannot be defined as a heterocaryotic fungus in the sense of Nelson *et al.* (13). However, as Buxton (6) has pointed out, uninucleate fungi need not be excluded from acting as heterocaryons, especially in the light of Pontecorvo's observations on parasexualism (15).

Anastomoses between hyphal strands have also been suggested as indicative of affinities between fungi (2, 4). Marked differences in the tendency to anastomose were observed between various strains of *M. populi* and attempts were made to derive possible associations from semiquantitative measurements of the intensity of interstrain anastomoses.

Methods

All of the isolates of *Marssonina populi* were obtained in 1958–59 during a survey of poplar diseases in the province of Quebec. Since it was not possible to isolate the fungi in the field, and dried or pressed specimens of diseased leaves from which the organism could be isolated were often heavily contaminated, the following method was used successfully. Small segments of the diseased leaf tissue were sterilized by shaking them continuously for 5 minutes in a vial containing 30% sodium hypochlorite and a drop of detergent. The segments were then rinsed once in sterile tap water and plated on acidified 2% water agar. The plates were then stored in a closed container (a large 48-oz

fruit juice tin sealed with aluminum foil and a rubber band). After 1 or 2 weeks abundant sporulation of the causal organism could be observed virtually free of any contamination. The spore masses could be picked off, made up to a suitable dilution in sterile tap water, and single-spore isolates made conveniently in the laboratory. The single-spore isolates were obtained with a small disk cutter and transferred to Difco PDA slants. Samples typical of each isolate were then refrigerated under mineral oil to maintain stock cultures. All cultures with the exception of the isolate from *Populus tremuloides* have been successfully maintained under oil for periods of at least 1 year without visible change in morphological characteristics, although a tendency to reduced sporulation was evident in some forms.

Growth studies were carried out in quadruplicate, in large (2-cm-diameter) test tubes in a controlled-temperature water-bath having an accuracy of approximately $\pm 0.1°$ C. Measurements of linear growth were made after 2 weeks to the nearest 0.5 mm. For observations on the effect of temperature on growth Difco PDA was used, but various other media were also employed as explained in the text.

To study anastomoses between biologic forms of *M. populi* "thread" cultures were employed. The organisms to be paired were grown in shake-culture in a 2% potato extract medium (Difco) in 150-ml Erlenmeyer flasks. To 20 ml of the medium containing a 15-cm length of light-weight thread, 1.0 ml of a calibrated spore suspension of 4×10^6 spores per ml was added. This concentration of spores was sufficient to completely cover the thread within 72 hours (Figs. 4, 5). At the end of this time the threads were lifted out with forceps, the visible growth stripped off aseptically, and the thread cut into 5-cm lengths. The different forms were then paired on 2% water agar, or occasionally PDA, at a distance of about 1.0 cm (Fig. 5). After 3 to 4 weeks at room temperature, at which time the mycelia from the threads had met, the cultures were examined.

Where evidence of anastomoses was observed two methods were used in attempts to obtain "hybrids" *in vitro*. Cultures were either paired on PDA by mixing a small sample of spores of the two forms together, or the actual anastomosed cells were lifted aseptically with a small micropipette (Fig. 5) and then transferred to PDA slants. In the latter case, a slightly different technique was employed to obtain anastomosed cells, because the density of growth in the normal "thread" culture method (Fig. 4) often made the isolation of single cells difficult. In this method threads 5.0 cm long were dipped in a concentrated spore suspension of the fungus and successive "prints" made by holding the thread taut between two forceps and then pressing it lightly on the surface of the agar. By this means it was possible to pair isolates separated by a distance of about 0.5 mm. When fusion was observed the anastomosed cells could be lifted with the micropipette, essentially free of other cells.

For cytological studies spores were germinated and grown on small squares of cellophane previously sterilized in 95% ethanol and placed on 2% water agar. While the cellophane impaired somewhat the quality of the photographic records it appeared to stimulate anastomoses. After a period of about 2 weeks the squares of cellophane with the germinated spores were lifted, partially dried, and fastened to slides with an albumin fixative. Hematoxylin and the

Feulgen reagent were used to stain the preparations. With hematoxylin, the slides were fixed in Craf IV (17) at 45° C for 4 hours, mordanted overnight using Heidenhain's formula (17), stained in an aqueous solution of hematoxylin, and de-stained in 0.1% mordant, the process being followed periodically under the microscope. This procedure proved satisfactory for spores but mycelial preparations could not be adequately de-stained. With the Feulgen reagent slides were fixed for 15 minutes at 60° C in Farmer's fluid (17) (3:1 absolute ethyl alcohol, glacial acetic acid). The fixative was then removed and the spore preparations hydrolyzed with 0.1 *N* HCl at 60° C for periods ranging from 10 to 30 minutes, the optimum time varying somewhat with different samples. The slides were immersed in the Feulgen reagent at room temperature for 1 hour (at which time maximum staining was attained), mounted in 45% acetic acid, and examined. Unhydrolyzed preparations were used as controls. A few permanent preparations were made and mounted in Canada balsam.

Since spore characteristics of the various races were not, under normal circumstances, sufficiently distinct to permit indiscriminate mixing of the spores, most cytological observations of anastomosed races were made only between one isolate from *Populus alba* × *grandidentata* and two others, one each from *P. deltoides* and *P. grandidentata*, by methods described in the text.

Pathogenicity trials were carried out in the greenhouse on rooted cuttings, or on small trees of several poplar species and hybrids. For the differentiation of races, spore suspensions were prepared and applied to the leaves with an atomizer. The leaves were then enclosed in a plastic bag (Fig. 6) and incubated for 72 hours. Symptoms developed on susceptible hosts within 10 to 24 days and isolations were then made from positive inoculations. All results have been confirmed at least twice over a 2-year period. Where large numbers of single-spore isolates (spores produced from anastomosed cells) were tested for evidence of hybridization on two hosts, each susceptible to one of the two "parents", the following adequately tested technique was employed (Fig. 3). Spore preparations were made aseptically from a portion of each culture to be tested and suspended in 0.5 ml of sterile tap water. Small squares of cheesecloth were cut, sterilized, and soaked in water. These were then placed on the leaves of the poplar to be tested (Fig. 6) and the free water removed with a strip of filter paper. Five-microliter quantities of the spore suspensions were then pipetted on the leaves, and the whole enclosed in a plastic bag and incubated in the

FIGS. 1–6. Fig. 1. Prepared slide of acervulus of *Marssonina populi* on *Populus deltoides* × "*Volga*" (stained with hematoxylin), ×500. Fig. 2. Leaves naturally infected with *M. populi*: from left to right in pairs, *P. deltoides* (Quebec City), *P. deltoides* (Berthierville), *P. balsamifera* (New Richmond), *P. alba* × *grandidentata* (Quebec City), *P. alba* var. *nivea* (Duchesnay), *P. tremuloides* (Quebec City), *P. pacheri* (Montreal), *P. angulata* × *simonii* (Calumet), *P. marilandica* (Calumet), *P. berolinensis* × *simonii* (Calumet), *P. deltoides* × *petrowoskyana* (Calumet). Fig. 3. Leaves of poplar inoculated in the greenhouse: from left to right in pairs, *P. balsamifera* (form G), *P. alba* var. *nivea* (form B), *P. tremuloides* (form F), *P. alba* × *grandidentata* (form F), *P. deltoides* (form D), *P. deltoides* (form G); the last two pairs were inoculated as illustrated in Fig. 6. Fig. 4. Paired cultures of form F (top and bottom) and form D (center); note inhibition zones. Fig. 5. "Thread" cultures of form G, uninoculated flask, left; inoculated flask after 72 hours, right; stripped threads after pairing, center; micropipette used in transferring anastomosed cells (above). Fig. 6. Inoculation of cuttings of *P. deltoides* with single-spore isolates of anastomosed cells of forms F and D of *M. populi*.

PLATE I

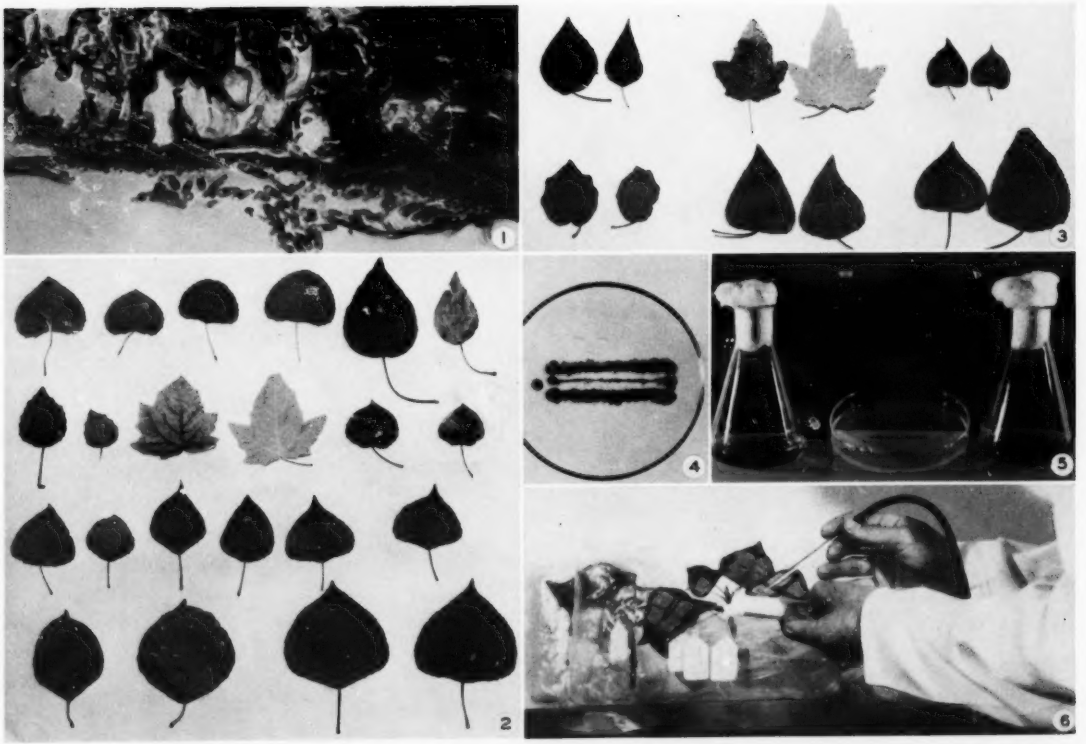

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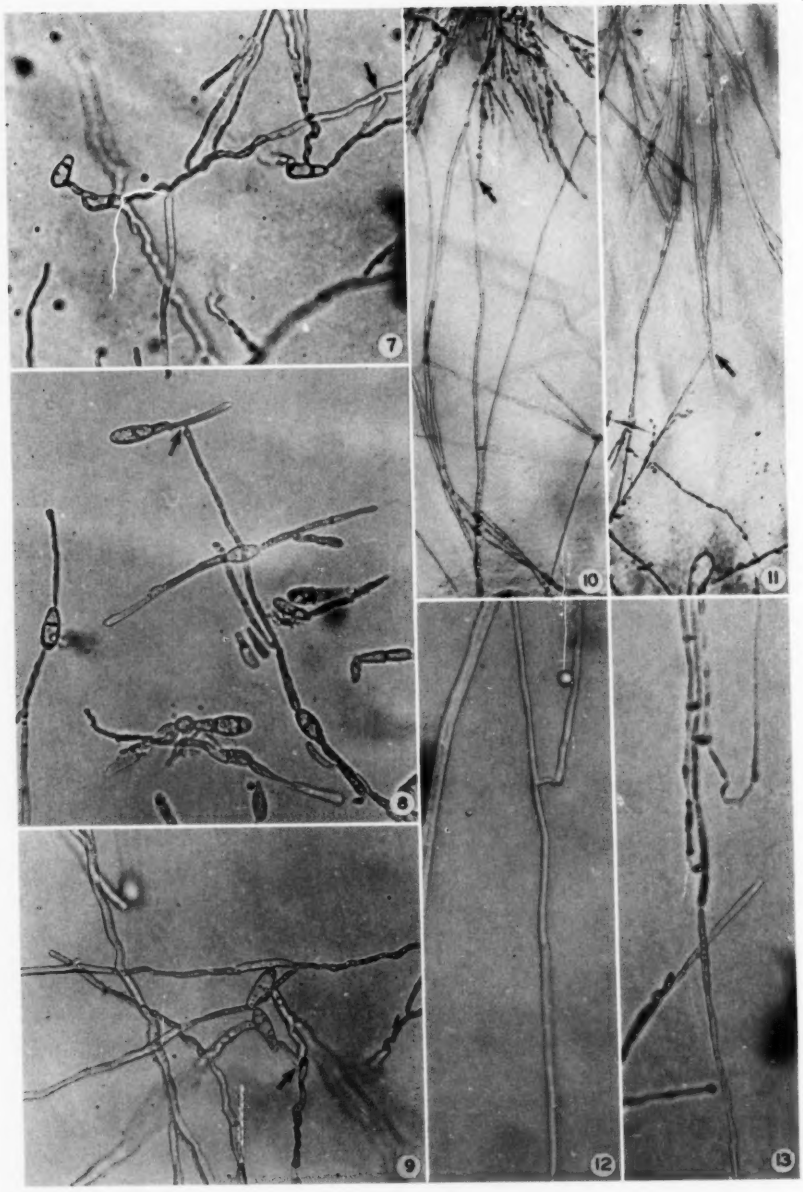
PLATE II



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dark at 22° C for 72 hours. At the same time a duplicate sample was plated on PDA to check viability. This method permitted the testing of 14 to 20 isolates per cutting. Generally, only one of the two hosts was inoculated with the intention of eliminating nonpathogenic isolates. Pathogenic forms could then be inoculated on the second host using the other half of each culture and, if they were pathogenic on this, evidence of hybridization could be assumed. More than 230 single-spore isolates were tested in this manner.

Results

Host Range and Symptoms of Marssonina populi on Poplars in the Province of Quebec

A large number of native and hybrid poplars are susceptible to the foliage disease caused by this fungus (Figs. 2, 3). The known hosts in Quebec are listed below:

Populus alba var. *nivea*
P. balsamifera
P. deltoides
P. grandidentata
P. tremuloides
P. alba × *grandidentata*
P. angulata × *simonii*
P. berolinensis × *simonii*
P. charkowiensis × *caudina*
P. charkowiensis × *incrassatta*
P. charkowiensis × "*robusta*"
P. deltoides × *petrowoskyana*
P. deltoides × *trichocarpa*
P. deltoides × "*Volga*"
P. eugenii
P. euramericana
Populus euramericana × "*serotina*" (Selys)
P. generosa
P. maximowiczii × *plantierensis*
P. maximowiczii × *trichocarpa*
P. nigra betulifolia × "*Volga*"
P. nigra "*italici*"
P. nigra × *laurifolia*
P. pacheri
P. petrowoskyana
P. raverdeau
P. sargentii
P. serotina
P. tremula
P. tremuloides × *tremula*
P. trichocarpa
P. trichocarpa × *tacamahaca*

While the distribution of *M. populi* is probably as widespread as that of its hosts, it is not prevalent in native stands. Only on *P. deltoides* have extensive infections been observed in Quebec. In other species, *P. balsamifera*, *P. grandidentata*, and *P. tremuloides*, severe infections appear to be localized, often affecting only one or two trees in a stand. This phenomenon has frequently been observed in mixed stands of hybrid poplars and is attributed either to a variation in resistance, or to host specificity on the part of the pathogen. However, since the organism has been shown to overwinter on twig lesions of at least one host, *P. deltoides* × "*Volga*", and the spores are disseminated primarily by rain, this might provide an alternative explanation for the heavy infestations observed in a limited number of trees.

On the leaves, petioles, and shoots of susceptible trees, infection is followed by the development of well-defined, brown, circular lesions, up to 1 mm in diameter. On more-resistant hosts the necrotic area is generally superficial and contained entirely on the side on which infection took place. On more-susceptible hosts the fungus may penetrate, and subsequently develop fructifica-

FIGS. 7–13. Fig. 7. Fusion between spores of form F, ×500. Fig. 8. Fusion between spores of form F (large spore) and form D (small spore), ×500. Fig. 9. Fusion between spores of form G, ×500. Fig. 10. Paired cultures, form D (above) and form F (below), ×100. Fig. 11. Paired cultures, form F (above) and form C (below), ×100. Fig. 12. Enlargement of anastomosed cells of Fig. 10, ×500. Fig. 13. Enlargement of anastomosed cells of Fig. 11, ×500.

tions on both sides of the leaf. Fruiting structures are formed at the center of the lesions as small, slightly raised acervuli, which appear white or yellowish white by reflected light because of the raised cuticular layers of the host. In older infections the lesions coalesce resulting in large, rust-brown or black necrotic areas on which the fungus sporulates abundantly. When infection becomes severe, complete or partial defoliation frequently occurs, commencing first at the base and progressing upward, that is, from the oldest to the youngest leaves of the host.

Origin and Morphology of Isolates

From more than 18 isolates of *M. populi*, 12 were selected for further study. Since only 3 of these have shown distinct and separate pathological characteristics, it is unlikely that all the existing forms or races of this organism have been obtained. However, the selections did provide a diverse cross-section of the pathogen from several taxonomically distinct hosts and geographical regions of Quebec (Table I).

While pronounced cultural and morphological variations occurred among forms (Fig. 36), their individual characteristics have remained essentially constant in culture, provided the forms were transferred regularly by spores. This manner of transfer was necessary, because some cultures often exhibited striking variations which to some extent were irreversible. A brief description of the morphological characteristics of each of the isolates is given below:

A—Colonies circular, flat, smooth, gray-brown in color with abrupt white to pinkish margins. Center slightly raised, with bristlelike conidiophores bearing few conidia. Spores from host, 12.0–18.0 μ by 3.6–6.0 μ (Fig. 15).

A_1—Colonies circular, flat, myceloid, margins fawn-colored. Center distinctly yellow with abundant spores produced on raised conidiophores often early becoming moist from dense accumulation of conidia. Spores from host, 12.0–21.6 μ by 3.6–7.2 μ (Fig. 16).

B, B_1—Colonies myceloid, flat, uniformly white or gray sometimes with a marginal faint pink halo. Whole surface covered with short, raised aerial mycelium rarely producing spores. Spores from host, 12.0–24.0 μ by 3.6–4.8 μ.

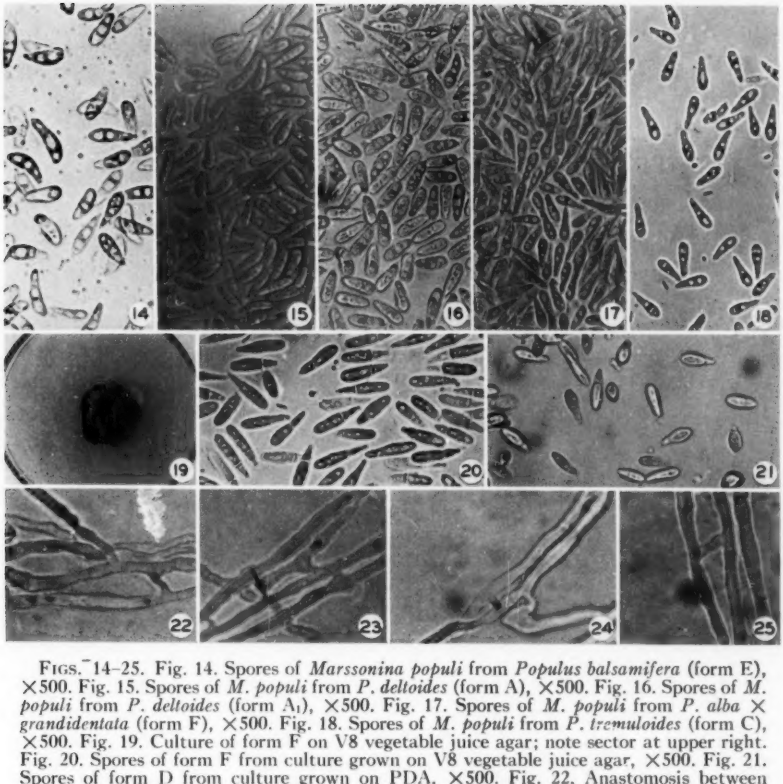
TABLE I

Origin of isolates of *Marssonina populi* studied

Code letter*	Host	Location
A	*P. deltoides*	Quebec City
A_1	*P. deltoides*	Berthierville
B	*P. alba* var. *nivea*	Duchesnay
B_1	*P. alba* var. *nivea*	Calumet
C	*P. tremuloides*	Quebec City
C_1	*P. tremuloides*	Lac St. Jean
D	*P. grandidentata*	Duchesnay
E	*P. balsamifera*	New Richmond
E_1	*P. balsamifera*	Quebec City
F	*P. alba* × *grandidentata*	Quebec City
G	*P. charkowiensis* × *robusta*	Quebec City
H	*P. deltoides* × *trichocarpa*	Quebec City

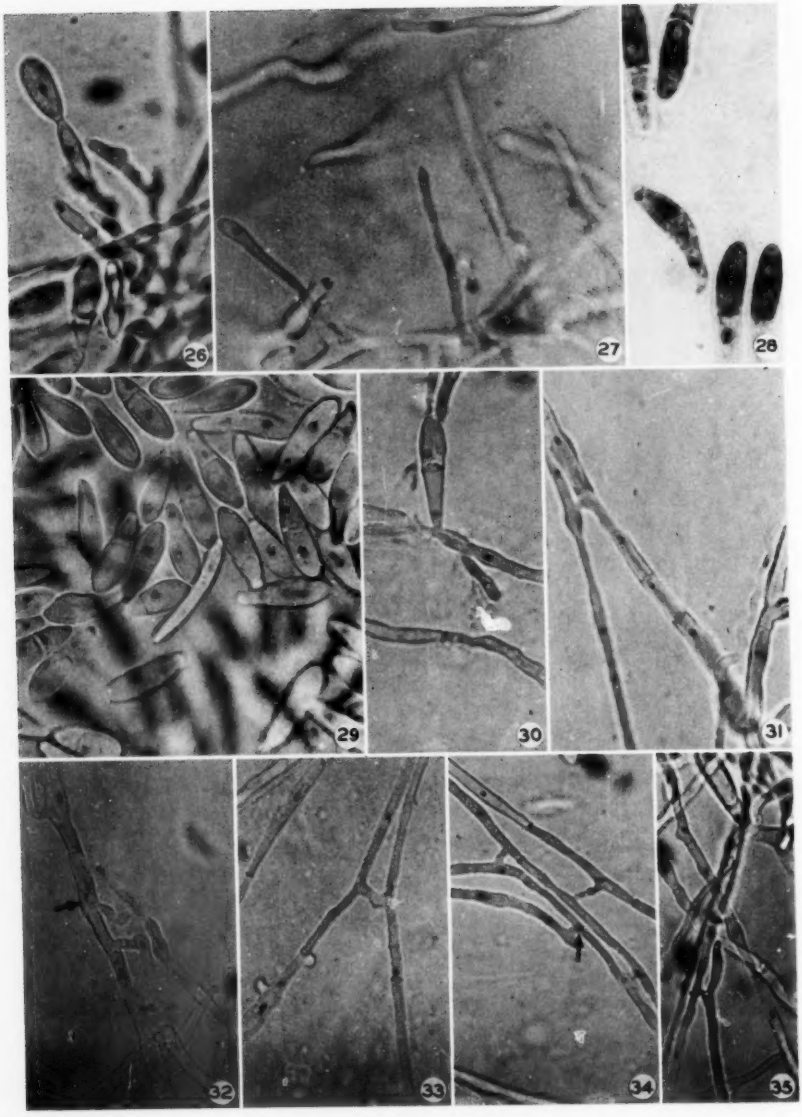
*These designations are used throughout the text.

PLATE III



FIGS. 14–25. Fig. 14. Spores of *Marssonina populi* from *Populus balsamifera* (form E), ×500. Fig. 15. Spores of *M. populi* from *P. deltoides* (form A), ×500. Fig. 16. Spores of *M. populi* from *P. deltoides* (form A_1), ×500. Fig. 17. Spores of *M. populi* from *P. alba* × *grandidentata* (form F), ×500. Fig. 18. Spores of *M. populi* from *P. tremuloides* (form C), ×500. Fig. 19. Culture of form F on V8 vegetable juice agar; note sector at upper right. Fig. 20. Spores of form F from culture grown on V8 vegetable juice agar, ×500. Fig. 21. Spores of form D from culture grown on PDA, ×500. Fig. 22. Anastomosis between mycelial cells of form F, ×1000. Fig. 23. Nuclear transfer from a hyphal-tip cell to an intercalary cell of form F, ×1000. Fig. 24. Anastomosis between mycelial cells of form F; nuclear fusion appeared to be taking place in the hyphal bridge, ×1000. Fig. 25. Nuclear transfer in anastomosed cells of form F; note the cross-wall in the bridging strand, ×1000.

PLATE IV



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C, C_1—Colonies flat, white margined with a faint yellow, sporulating center. Spores produced abundantly on raised conidiophores frequently accumulating in moist bright yellow yeastlike masses. Spores from host, 12.0–19.2 μ by 3.6–4.8 μ (Fig. 18).

D—Colonies flat, smooth, white with gray margins early turning yellow at center. Spores borne over whole surface at first not abundantly. Spores from host, 12.0–19.0 μ by 4.8–6.0 μ.

E—Colonies white to gray with a dense covering of very long aerial mycelium clinging together in moist clumps. Normal spores produced rarely, superseded by atypical extremely long, narrow, two-celled spores of low viability, or colonies sterile. Spores from host, 16.8–26.4 μ by 4.2–8.4 μ (Fig. 14).

E_1—Colonies smooth, faint red with gray margins. Central portion becoming moist yellow to yellow-green with dense covering of spores. Spores from host, 12.0–22.8 μ by 4.2–6.0 μ.

F—Colonies slightly raised, irregular, fawn-colored to gray, often zonate, with faint yellow center densely covered with short bristlelike conidiophores. Central part of colonies early become yeastlike and distinctly yellow with accumulated masses of spores. Spores from host, 10.8–20.4 μ by 3.6–4.8 μ (Fig. 17).

G—Colonies raised, myceloid, at first white then turning faint red or rust-colored at center, gray to white at margins. Spores generally produced centrally in small, moist, fawn-colored clusters. Spores from host, 13.2–22.8 μ by 4.2–7.2 μ.

H—Colonies distinctly smaller, myceloid, gray to faint yellow, erumpent and folded. Spores not produced abundantly, found sparsely over whole surface. From host, 12.0–18.0 μ by 3.6–6.0 μ.

To further characterize each form, spore suspensions were prepared from the host and from 2-week-old PDA cultures and random samples of 100 spores measured, excluding immature or malformed spores. The distribution of spore lengths by classes was determined (Table II) from these measurements.

As suggested by the data, spore size and distribution, at least in part, respond to environmental conditions, and probably from that standpoint alone cannot be safely used in a taxonomic characterization of the forms. Neither does the length distribution of spores adequately separate the forms (see Table II, A_1, C, D, F). To determine the influence of the host on spore characteristics, forms A, D, and G, which were shown to be parasitic on *P. deltoides*, and forms F and C parasitic on *P. tremuloides* were inoculated on

FIGS. 26–35. Fig. 26. Nuclei in hyphal tips and conidium of form B, ×1000. Fig. 27. Nuclei in the hyphal tips of form C, ×1000. Fig. 28. Nuclei in mature spores of form F (from V8 vegetable juice agar) stained with hematoxylin, ×1000. Fig. 29. Nuclei in mature spores of form F (from PDA) stained with the Feulgen reagent, ×1000. Fig. 30. Nuclei in a germinated spore of form F, ×1000. Fig. 31. Anastomosis between forms D (right) and F (left); note position of nuclei, ×1000. Fig. 32. Apparent nuclear transfer from form D (right) to form F (left); note presence of cross-wall in hyphal bridge, ×1000. Fig. 33. Transfer of the nucleus from form F (left) to form D (right), ×1000. Fig. 34. Hyphal fusions between and within forms D (upper two strands) and form F (lower two strands); the nucleus from the intercalary cell of form F was apparently in the process of migration into the hyphal tip (indicated by arrow), ×1000. Fig. 35. Anastomosis between forms D (right) and F (left); no nucleus is apparent in the short cell of form F while the intercalary cell of D appears to be binucleate.

TABLE II

Percentage distribution of spore lengths of isolates of *Marssonina populi*

Isolate	Origin of spores	Micrometer units*														
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A	Host				2	18	40	27	7	4	2					
	PDA				7	3	8	9	37	20	13	3				
A_1	Host				12	3	34	18	25	8						
	PDA			9	0	1	19	22	32	7	8	2				
B	Host					4	6	5	19	32	17	5	6	3	3	
	PDA					5	6	2	17	30	27	11	2			
B_1	Host					6	7	12	21	33	15	4	2			
	PDA					2	4	6	18	25	30	12	3			
C	Host				5	5	35	15	34	3	3					
	PDA			2	5	8	20	18	32	0	0	5	7	3		
C_1	Host				3	2	35	18	32	7	3					
	PDA					9	22	10	40	5	0	9	5			
D	Host			2	5	5	22	20	28	11	7					
	PDA		3	0	7	23	23	26	7	7	2	2				
E	Host					8	5	8	12	15	30	16	1	5		
	PDA						4	8	8	10	13	34	14	8	1	
E_1	Host			3	5	5	30	15	20	12	5	5				
	PDA			6	12	14	20	24	16	8						
F	Host		5	5	10	27	10	19	10	11	3					
	PDA	3	2	3	7	19	12	33	5	3	3	5	2	3		
G	Host			3	4	1	40	15	18	9	7	3				
	PDA			1	4	9	17	27	16	10	4	12				
H	Host			3	32	13	34	13	5							
	PDA		4	3	4	2	8	9	4	9	14	27	13	2	1	

*To convert to μ multiply by 1.2.

areas of the same leaf of their respective hosts in the greenhouse. After 1 month, random spore samples were obtained and the distribution of spore-lengths again calculated. Form A, isolated originally from *P. deltoides*, retained its characteristic distribution while forms C, D, F, and G did not. Thus, it was concluded that neither spore size nor distribution can be used to differentiate between isolates.

Pathogenicity of Isolates

Five species and three hybrid poplars were used to obtain an indication of the host range of the forms under study. These did not clearly differentiate between all forms, but data were obtained which indicated that at least five biologic forms of *Marssonina populi* are prevalent in Quebec (Table III).

Forms B, C, and F are clearly distinct. Forms A_1 and D and A, G, and H compose two groups which cannot be separated further with the limited number of poplars used. An interesting result was obtained when two forms parasitic on *Populus deltoides* were isolated. Form A was found to be parasitic on *P. balsamifera* in addition to its original host, while A_1 was parasitic on *P. deltoides*, *P. grandidentata*, and *P. balsamifera*. Both isolates of *P. alba* var.

FIG. 36. Isolates of *Marssonina populi*, from left to right: forms C, C_1, A, D, H, G, B, B_1, E, and F. Note sectoring in A, D, G, and E.

FIGS. 37–40. Single-spore isolates (second transfer) obtained from anastomosed cells of forms F and D. Note uniformity of isolates. The zonate growth evident in all the cultures was typical of form F.

PLATE V

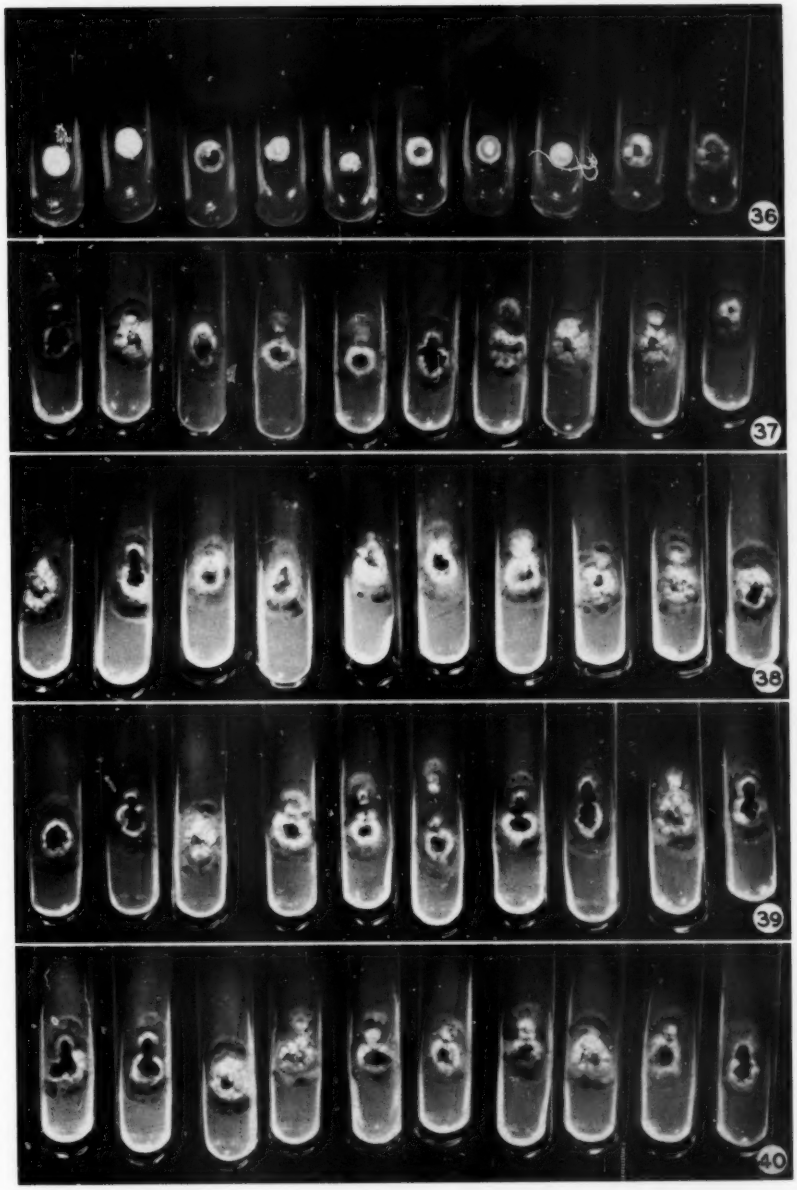

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TABLE III

Pathogenicity of isolates of *Marssonina populi* on poplars

Isolate	Poplar species or hybrid*							
	deltoides	*alba*	*tremuloides*	*grandidentata*	*balsamifera*	*alba* × *grandidentata*	*charkowiensis* × "*robusta*"	*deltoides* × *trichocarpa*
A	X				X		NT†	NT
A_1	X			X	X		NT	NT
B		X					NT	NT
B_1		X					NT	NT
C			X	X			NT	NT
C_1			X	X			NT	NT
D	X			X	X		NT	NT
E_1	X				X		NT	NT
F			X	X		X	NT	NT
G	X				X		X	X
H	X				X		X	X

*Inoculations were carried out on mature leaves.
†NT = not tested.

nivea (B, B_1) were restricted to this host, while those from *P. tremuloides* (C, C_1) were parasitic on both *P. tremuloides* and *P. grandidentata*. Form F, parasitic on *P. alba* × *grandidentata*, was pathogenic on *P. tremuloides* and *P. grandidentata*, but not on *P. alba* var. *nivea*. This isolate subsequently proved to be unique. Morphologically and pathogenically it appeared to be closely related to form C. On its original host, *P. alba* × *grandidentata*, this form is only a weak parasite, yet it is strongly pathogenic on *P. tremuloides* under greenhouse conditions. Evidence, which will be presented later, led to the interpretation that this pathogen was a mutant derived from a form originally parasitic on *P. tremuloides*.

Anatomical features of the host probably do not play an important role in the susceptibility or resistance to specific forms of a pathogen. Among anatomical variations observed in the poplars used in these pathogenicity trials was the location of the stomata. On native poplars the stomata are confined to the lower side of the leaf, while in many introduced poplars they are amphigenous. To test the possibility that this or other anatomical differences might act in preventing infection, cut branches from the poplars used in the trials were placed in water and inoculated with at least one of the normally incompatible isolates. In all cases, symptoms developed within 2 weeks, which suggests that resistance is attributable to physiological rather than anatomical differences.

Cultural Studies

At least one isolate from each host was examined culturally for evidence of similarities or differences. Optimum temperatures were determined by measuring the colony diameter of the organisms after 14 days' growth on PDA slants in a controlled-temperature water-bath. Quadruplicate tubes were inoculated with a small quantity of spores on the tip of a wire needle. Since age and the medium on which the inoculum was grown greatly influenced the subsequent growth rate, approximately 2-week-old cultures on PDA were used as a source of spores for inoculation. Some variation among the isolates was evident with respect to the effect of temperature on growth (Fig. 41).

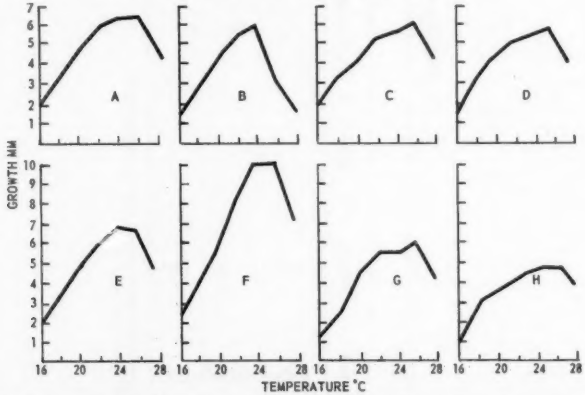


FIG. 41. Linear growth of isolates of *Marssonina populi* as affected by temperature.

Form F from *P. alba* × *grandidentata* exhibited a distinctly greater growth response at all temperatures, but had an optimum temperature within the range 24–26° C in common with all other isolates. Form B from *P. alba* var. *nivea* had a narrower optimum temperature range than the other isolates with a maximum growth rate at 24° C. With the exception of form H, which showed a distinctly slower growth rate at all temperatures, the remainder responded in a similar manner to temperature changes.

Several other media (malt agar, V8 vegetable juice agar, and agars prepared from several leaf extracts) were used with the isolates to determine the best medium to support abundant sporulation and to determine whether any evidence of growth inhibition or acceleration was evident on leaf extracts of compatible or incompatible hosts. None of the media tested supported either growth or sporulation as satisfactorily as did PDA. In addition, no variation in response to temperature from that obtained on PDA was evident. However, the comparative growth rates among isolates on the various leaf extracts demonstrated some differences.

In general, leaf extracts did not support abundant growth of the organisms. Extracts prepared from *P. deltoides*, *P. alba*, and *P. alba* × *grandidentata* were uniformly poor. On the other hand, preparations from *P. balsamifera* supported growth comparable with that obtained on malt or V8 juice agars for the forms E and F while suppressing the growth of the other isolates, suggesting that some extracts contain toxic substances, or are deficient in nutrients, to specific isolates of *M. populi*. Preference for their own host extract was not characteristic of any of the forms other than E.

Hyphal Anastomoses among Isolates of Marssonina populi

During preliminary studies on spore germination of form F of *M. populi* it was observed that in cultures held for 2 weeks or more, anastomoses were common between mycelial strands from single spores (intraspore) and from two or more spores (interspore) (Figs. 7–9). Frequently, intraspore fusions resulted in a "ladderlike" effect uniting mycelial strands where these grew in close proximity to each other. While characteristically only one hyphal "bridge" linked two adjacent cells of paired strands, two or often three were sometimes observed. Hyphal tip fusions were rarely seen, the frequency of anastomoses increasing in the morphologically older parts of the mycelium. Normal growth from a single spore radiated "fanlike" from one or more germ tubes. Between adjacent strands of this mycelium the extreme interhyphal distance at which anastomosis took place was approximately 30 μ. Beyond this distance fusion did not occur. This measurement was based on the observation that, in most cases, mycelium linking adjacent cells of the hyphae arose at an angle of 90° to the parent strand, whereas random hyphal growth was always at less than 90° (Figs. 10–13).

Anastomoses between and within mycelial strands of a culture derived from a single spore suggest a vegetative rather than a sexual function. It was important therefore to ascertain whether such a process, which could lead to diploidization, was necessary for the development of the disease. From dilution series of spores from form F of *M. populi* plated on 2% water agar in Petri dishes, single spores were lifted with a small disk cutter and placed on leaves

of *P. alba* × *grandidentata*. After 3 weeks' incubation 25 lesions were recorded from a total of 86 single-spore inoculations. From some of these the fungus was reisolated. This led to the conclusion that single spores are pathogenic and that interspore anastomoses were not necessary for the development of infection.

The ability of different forms of *M. populi* to anastomose with each other was also investigated. Liquid cultures each containing a 15-cm length of thread were prepared and inoculated with a standardized spore suspension of the forms A, B, C, D, E, F, G, and H. The forms were then paired as described previously. The line of contact between forms was examined closely (Figs. 10–13) and counts of anastomosed cells were made (Table IV).

TABLE IV

Anastomoses between paired biologic forms of *Marssonina populi*

Form	A	B	C	D	E	F	G	H
A	XX		X	X	X	XX	X	X
B		X				X		
C			XX	X	X	XXX	X	X
D				XX	X	XXX	X	X
E					XX	XX	X	X
F						XXX	X	X
G							X	X
H								X

NOTE: X = less than 5 per plate; XX = 5–19; XXX = 20 or more. Values are not strictly comparable since they are influenced to some extent by density and rate of growth.

It is evident from these data that hyphal anastomoses do occur between biologic forms of *M. populi*, although only in the case of cultures paired with form F did interform quantitatively exceed intraform unions. This was due in part to the increased probability of the event occurring because of the comparatively rapid growth of this form. Form B, with a low frequency of intraform hyphal fusions, anastomosed only rarely with form F, while nearly all other isolates showed little or no specificity. Forms F, D, and C appeared to unite more frequently and earlier than other isolates and this together with certain morphological similarities suggested that a possible closer relationship existed among these three forms than among any of the others. Form B, on the other hand, with dissimilar morphological and cultural characteristics also exhibited no tendency to anastomose with forms other than F.

Mutual inhibition was consistently observed in forms of *M. populi*. For example, if thread cultures of distinct races, or of the same race, were paired, a pronounced but localized zone of inhibition became evident between the two (Fig. 4). Whatever the cause it was nonspecific, affecting all isolates of *Marssonina* without exception. This phenomenon was not studied in detail but the existence of an inhibitory factor opposite in effect to the positive stimulus of anastomosis posed a rather interesting problem. Observations on paired cultures of forms F and D, both alone and together, suggested a partial explanation. Unions between and within these forms are primarily, yet not entirely, a function of cells located some distance from the growing point. In most cases the two participating cells were more than 144 μ from their respective growing points, although frequently anastomosis occurred between the terminal cell of

one hyphal strand and an intercalary cell of another (Fig. 12). Since, under the conditions of the experiment, abundant opportunity for cell fusion existed between terminal cells or those immediately adjacent to them, it is suggested that the inhibitory factor is specifically located in or near the growing point, while the phenomenon of anastomosis appeared to be a function of older cells.

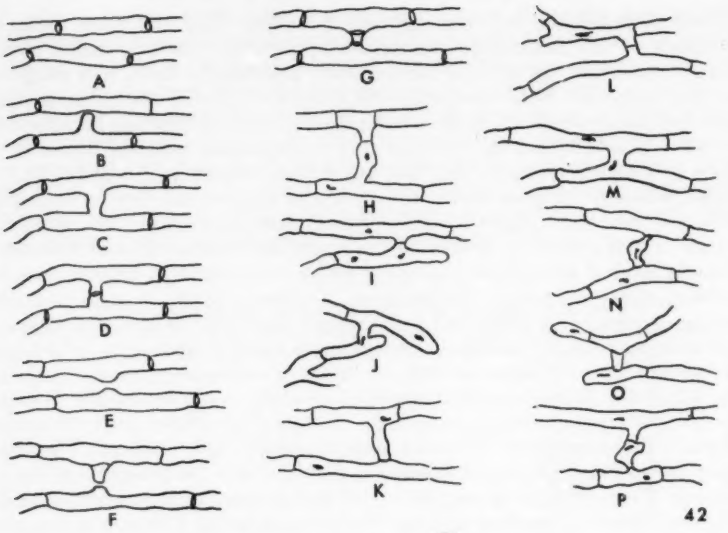
While Table IV lists the frequency of anastomoses between forms of *M. populi* it is highly probable that not all of them represented true hyphal cell fusions, whereby, at least during some stage of the process, free intermingling of the cell contents occurred. Observations were made on paired cultures of forms F, and F and D, to determine the origin and time of cell-wall formation. Adjacent hyphal strands were selected where the process of anastomosis had commenced as indicated by the initiation of a lateral hyphal branch (a marked swelling or protrusion of the cell wall at right angles to the strands). Observations were made over a period of several hours, using a microscope at a magnification of 450×. At least two distinct types of anastomoses were recognized (Fig. 42, A–G). In the most frequent type (Fig. 42, A–D) the hyphal "bridge" was initiated from one of the two parent strands. This strand, prior to contact, induced a slight indentation in the wall of the receiving hyphal cell. The actual fusion process was difficult to observe in detail as was the behavior of the cell contents. The process from contact to cell-wall dissolution was short, however, being completed in less than an hour. Within a period of 12 hours, a new cross wall had formed. In other examples no wall formation occurred after dissolution and therefore free movement from one cell to another was possible over a more extended period. Because this condition was observed much less frequently in studies on anastomoses, it may possibly represent only an intermediate stage of the first process, cell-wall formation within the bridging strand being delayed for a period longer than the process could be observed. Mutual fusion between two component strands also took place (Fig. 42, E–G). In this case no apparent dissolution of the original walls occurred and in addition another wall was laid down within the bridging strand leaving a central cell. Subsequent observations on the distribution of nuclei in anastomosed cells (Fig. 42, H–P) could be related to these two types. The important conclusion derived from this study was that the presence of one or more cross walls within the bridging strand linking two cells does not in itself preclude the occurrence of nuclear migrations or the mixing of other cell components.

Nuclear Observations

For cytological studies, spores were germinated on cellophane and stained with the Feulgen reagent, or with hematoxylin, as described earlier.

To study anastomoses cytologically, mixtures of spores of selected forms were made. Separation of a mixture of spores of two or more races, either by size or by germination characteristics, was not always possible. However, form F when grown on one-half strength V8 vegetable juice agar unadjusted as to pH (Fig. 19) yielded a great abundance of highly viable spores, much longer than the other races grown on PDA[3] (Figs. 20, 21). This provided a useful characteristic for the separation of this form from some of the others. When two

[3]The remaining forms failed to grow under these conditions. When the pH was adjusted to 6.5, some growth resulted but sporulation was completely suppressed.



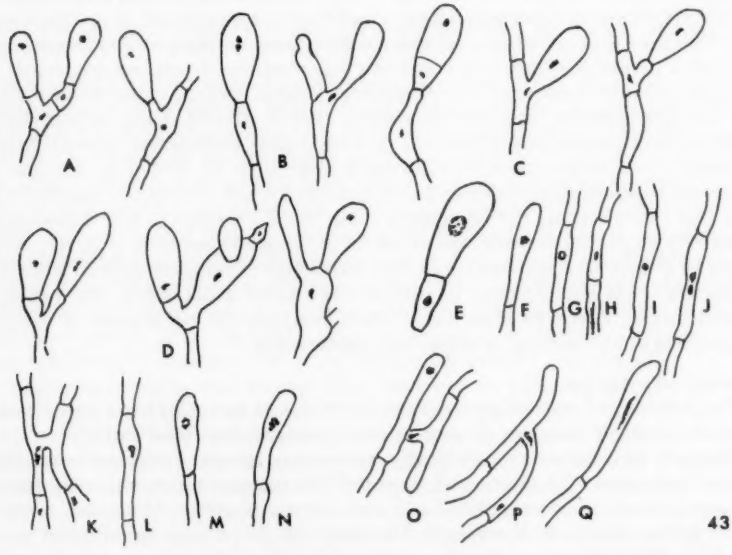


FIG. 42. Anastomoses in cultures of *Marssonina populi:* A–D, sequence 24 hours, between form F (lower) and form D (upper); E–G, sequence 10 hours, form F; and H–P, nuclear configurations arising from anastomoses. All ×2000.

FIG. 43. Nuclear configurations in conidia and conidiophores of various forms of *M. populi* stained with the Feulgen reagent: A, form F; B, form C; C, form A_1; D, form D, and nuclear configurations in spores and mycelium of form F of *M. populi;* E, a nongerminated spore showing the diffuse type of nucleus; F–Q, nuclei of intercalary and terminal cells, ×2000.

races were mixed a sample of each was prepared separately and estimations made of spore length after treatment with the Feulgen reagent.

The normal number of Feulgen-positive bodies (nuclei) per cell of the mycelium was one for all of the races (Figs. 26–35). Even in hyphal tips this number was generally observed. However, occasional terminal and intercalary cells (less than 0.01%) were found with two or three nuclei. Since the phenomena of nuclear division and cross-wall formation appeared to be chronologically distinct events, it was assumed that the occurrence of multinucleate cells represented stages between these two processes. Other cells were also observed devoid of stained nuclei and, as these were seen often randomly located in linear series of cells containing prominent nuclei, it was concluded that in some cells they were absent. This appeared to be true of some of the short cells (Fig. 24) often involved in anastomosis. For this reason it was not possible to state unequivocally that nuclear fusion had occurred in uninucleate anastomosed cells.

Nuclei in many stages of development were observed. The apical cell of the nongerminated spore contained a large, centrally located, and diffusely stained body (Figs. 28, 29). The basal cell contained a morphologically distinct nucleus which was smaller, generally located randomly in the cell, and stained more intensely than the apical cell nucleus. The origin of these nuclei could be discerned in stained preparations. In all cases both nuclei of the spore arose from a single division within the walled-off, unicellular spore (Figs. 26; 43, A–D) and were therefore probably genetically identical, in spite of differences in appearance. After germination the nuclear structure of the spore was radically altered. Both nuclei became much smaller and stained more intensely than the nuclei in the nongerminated cell (Fig. 30). The actual process of germination was not followed closely, other than to determine that both cells may germinate and contribute their nuclear content to the mycelium (Figs. 7–9). The mycelia and nuclei from both the apical and the basal cells appeared morphologically identical and functioned freely in anastomoses. Nuclei within the mycelial cells varied considerably in appearance (Fig. 43, E–Q). The nuclear structure observed most frequently was a small diffusely stained body, highly variable in shape (Figs. 32, 35, 43). Other nuclear forms leading to division based on those observed in form F of *M. populi* could be recognized (Fig. 43). As the nucleus approached division the Feulgen-positive substance formed a ringlike structure at the periphery of the nucleus (Fig. 43, F, G). Marked contraction then took place (Fig. 43, H) followed by division (Fig. 43, I). Reversion to a diffuse-staining type of nucleus appeared to be rapid (Fig. 43, J).

Observations on nuclear changes in anastomosed cells were made with mixtures of the biologic forms D and F (Figs. 31, 32, 33, 34, 35), and within forms F (Figs. 22–25). All the forms examined anastomosed freely on the cellophane strips and no striking differences in nuclear behavior were evident between inter- and intra-form hyphal fusions. In both types dicaryotization appeared to result infrequently (Figs. 23, 32, 33) and, more rarely, there appeared to be nuclear fusion (Fig. 24).

However, anastomosis between hyphal cells of two strains appeared to be a purely vegetative process which only infrequently led to dicaryotization and

TABLE V

Position of nuclei in anastomosed cells of two forms of *Marssonina populi**

No. of septae in anastomosed cells	No. observed	Component nuclei separate	Component nuclei together	Single nucleus only observed	Nucleus in bridging strand
No septum	20	16	2	2	0
One septum	50	48	1	1	0
Two septa	42	40	0	1	2

*Includes data from both inter- and intra-form anastomoses.

nuclear fusion, and then probably only by chance. The frequency data from one sample of a mixture of forms F and D (Table IV) given in Table V supported this interpretation.

From Table V, of a total of 112 anastomosed cells, only two resulted in the probable transfer of a nucleus from one cell to the other. Where one or two septae are formed, nuclear transfer is apparently impeded to some extent, but not entirely (Figs. 32, 33).

The occurrence of uninucleate anastomosed cells (Figs. 22, 31) is obviously of interest, since it implies the possibility of nuclear migration followed by fusion. However, as pointed out previously, this interpretation is open to question because of the apparent existence of cells in the hyphae without nuclei or with nuclei which did not stain with the Feulgen reagent. The approximate nuclear diameter taken from uninucleate mycelial cells was 1.2 μ, almost identical with the dimensions observed for most uninucleate anastomosed cells although nuclear dimensions varied widely (Fig. 43). However, oversized nuclei have been observed infrequently (Fig. 23), which would suggest that fusion had occurred. If such a mechanism is not involved in the proposed asexual formation of hybrid forms it is difficult to visualize any means by which such a phenomenon could take place, for no evidence of duplication of a binucleate condition through renewed growth of anastomosed cells was ever observed.

Pathogenicity of Selections from Anastomosed Cells of Biologic Forms

Three biologic forms were selected for subsequent studies on vegetative hybridization: F from *Populus alba* × *grandidentata*, A_1 from *P. deltoides*, and D from *P. grandidentata*. Each of the latter two was crossed with the former. Evidence of hybridization was to be based on the isolation of single spores from paired matings, which on subsequent inoculation would be pathogenic on two hosts, each of which was susceptible to only one of the parent fungi. The two hosts used in this study were *P. deltoides* on which both A_1 and G were pathogenic, and *P. tremuloides* on which form F was pathogenic. Crosses were first attempted by mixing a few spores of each of the two forms singly with a sample of spores from form F. These were transferred at 2-week intervals for four successive transfers. At the end of this period five test tubes of the mixed cultures F and A_1 were combined in one and grown for a further 2-week period. Spore dilutions were made, plated on 2% water agar, and 100 single-spore isolates were made. The same procedure was followed for the

mixed culture F and G. From the former, 78 viable single-spore cultures were obtained, from the latter 81. The single-spore isolates were then tested individually on established cuttings of *P. deltoides*. Without exception none were pathogenic on this host whereas a random selection of 20 cultures were all pathogenic on *P. tremuloides*. These results coupled with morphological observations (Figs. 37–40) indicated that only form F type cultures were among the isolates.

The results were not entirely unexpected in view of the observation of inhibition in culture previously discussed. In this case the faster-growing, more-vigorous isolate appeared to assume dominance in mixed culture. Another method, using isolated individual anastomosed cells, was then tried. In this experiment only forms F and D were paired. Twenty anastomosed cells in which no cross wall had formed were transferred by means of the micropipette to PDA slants. Of these, 18 were viable. After 2 weeks' growth spore dilutions were prepared, mixed, and 100 single-spore transfers made. The 81 viable cultures were inoculated on cuttings of *P. deltoides*. Two of these were pathogenic on this poplar, but subsequently were shown to be nonpathogenic on *P. tremuloides*. If in the anastomosed cells of these two races nuclear transfer occurred, then it would appear that it did not result in hybridization. While the failure to obtain any evidence of hybridization in anastomosed cultures by these methods cannot be taken as proof that this phenomenon does not occur *in vivo* or *in vitro*, the morphological, cytological, and pathological observations on isolates of *M. populi* made here would lead to the interpretation that new forms of this organism do not arise as a result of anastomoses between existing forms.

The Possible Origin of Forms of Marssonina populi

Two further possible sources of variation exist in *M. populi*, and their potential importance has not been fully investigated. Hybridization through the sexual stage, *Drepanopeziza populorum*, is a possibility, particularly under conditions existing in the plantations where several forms on distinct hosts exist in close proximity. In Quebec, the perfect stage of this fungus has been observed on overwintered leaves of *P. grandidentata*, *P. deltoides*, and *P. charkowiensis* × "*robusta*". From the hybrid, single-ascospore isolates have been made and the resulting cultures linked with the imperfect stage. While these have been the only forms examined, it would appear that the perfect stage of *M. populi* is widely distributed.

Mutations may also be important as a cause of variation in this fungus. Although it has been possible to maintain most cultures in a stable condition by using spore rather than mycelial transfers, the fungus appeared to be highly variable in culture, sectoring frequently. Whether such changes are typical of the organism *in vivo* is open to question, but the diversity and multiplicity of forms obtained would certainly imply that such is the case.

The unique properties of form F are suggestive of changes arising through mutation rather than hybridization. If the form is a mutant, a logical parent form would have been the pathogen on *P. alba* or *P. grandidentata*. Various experiments were undertaken to examine similarities among forms B, C, D, and F. Among these, the effect of pH on germination and sensitivity of spores

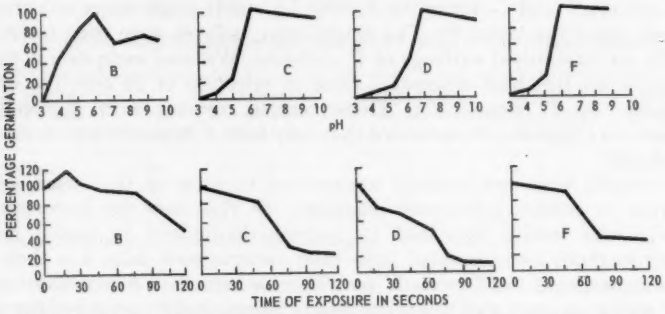


FIG. 44. The effect of pH and exposure to ultraviolet light on the germination of spores of isolates of *Marssonina populi*.

exposed to ultraviolet light (Fig. 44) yielded interesting results. From these observations and the results of pathogenicity trials, form F would appear to be unrelated to form B. While it has many characteristics in common with both forms D and C, pathologically it is more closely related to C, and it is suggested that form F is an isolate derived originally from one pathogenic on *P. tremuloides*.

Discussion and Conclusions

Critical studies of several biologic forms of *Marssonina* parasitic on species and hybrids of poplar were undertaken and morphological, pathological, and cultural characteristics compared. As a group, with the exception of form F, the isolates appeared to consist of a series of closely related fungi often separable on both pathological and cultural bases but too highly variable to be distinguished clearly taxonomically. In this respect the cultural data generally supported the taxonomic interpretation of Thompson (19), that is, that no sufficiently distinct criteria existed for the separation of the forms into species. In minor qualitative characteristics form B would appear to differ, but whether these considerations are important enough to separate this isolate taxonomically is doubtful.

Hyphal anastomosis was a commonly observed phenomenon in the forms examined. Its possible importance as a means whereby variation could arise was investigated, but no evidence in support of this was obtained. Cytological observations indicated that the fungus was uninucleate. As a result of anastomoses, dicaryotization took place infrequently but the binucleate condition was not perpetuated. Fragmentary evidence suggested that nuclear fusion takes place occasionally under the same circumstances. If this is so, it is possible that exchange of nuclear material could take place resulting in new races with characteristics intermediate between the two parents. Other than in this manner, there was no apparent way in which anastomoses could play a role in the development of new forms.

Some evidence obtained in this study points to mutations rather than hybridization as the primary source of variation among the forms of *M. populi*. At least one, form F, exhibiting no characteristics intermediate between

possible parent forms, appeared to be a recent mutant pathogenic on a newly introduced hybrid poplar. In addition, the distinct cultural diversity among and within the forms of this uninucleate fungus, and their instability, strongly supported this interpretation.

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SPORE DISPERSAL IN POLLACCIA RADIOSA (LIB.) BALD. AND CIF.[1]

B. W. DANCE[2]

Abstract

The effects of environmental and silvicultural factors on the dissemination and concentration of spores of *Pollaccia radiosa* (Lib.) Bald. and Cif. in the air were studied at Chalk River, Ontario. Relationships were found to exist between the concentration of spores in the air, rain showers, the time of year, and the susceptibility of the host(s); a knowledge of these may be valuable in controlling the pathogen.

Introduction

In Canada, *Pollaccia radiosa* is perennially present on trembling aspen (*Populus tremuloides* Michx.), large-toothed aspen (*P. grandidentata* Michx.), and on various introduced species and varieties of the section *Leuce* Duby (3). It has been observed by the author that the pathogenic capability of this fungus varies with different hosts, and within one species or clone according to the age of individual specimens; larger trees may be relatively unaffected, while seedlings and suckers generally are severely attacked. This results in the loss of established regeneration, in the deformation of saplings, and in a delay of one or more years in establishing new stands.

It is evident, therefore, that the activity of *P. radiosa* constitutes a problem for tree breeders seeking to create new hybrids which are easily propagated, fast-growing, disease-resistant, and superior to existing species and varieties in silvicultural characteristics. Hence, new information which might help control the depredations of *P. radiosa* would be valuable.

This investigation was designed to clarify the role of environmental factors affecting spore dispersal in *P. radiosa*. The experimental work was conducted during the summer of 1957 at the Petawawa Forest Experiment Station, Chalk River, Ontario, where a suitable site for collecting data existed.

The Disease

Primary infections of the imperfect fungus, *Pollaccia radiosa*, first appear about mid-May on trembling aspen leaves, and later on large-toothed aspen, in the immediate vicinity of blighted, overwintered shoots. Observations indicate they are initiated by conidia (Figs. 4 and 5) produced by mycelia in the blighted shoots, and by ascospores of *Venturia tremulae* Aderh., the ascigerous stage of the fungus (1), which occasionally develops on these shoots. Secondary infections appear in late May on leaves and shoots situated anywhere on the plants. Apart from their different origin and unrestricted distribution, these appear similar to primary infections. Subsequently, the numbers of infections multiply rapidly until by late spring most shoots and

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leaves of aspen regeneration are affected. The symptoms consist mainly of dieback, curling, and blackening of new terminal shoots; and hypotrophy, distortion, and necrosis of leaves on these (Fig. 1). Variable-sized, separate or coalescent spots develop on living leaves (Fig. 2). An olive-green layer of 1- to 4-celled (usually 2-celled) spindle-shaped conidia forms in places over necrotic leaf tissue (Figs. 1 and 2).

Materials and Methods

The study was conducted in a densely stocked, 2-year-old stand of trembling aspen which, by June 6, 1957, was heavily infected with *Pollaccia radiosa*. Infected specimens of large-toothed aspen were prevalent in the vicinity. The diseased plants had become established after a clear-cutting operation in mixed hardwoods. The median height was about 5 ft, but occasional individuals up to 10 ft tall were present.

Four spore-trapping stations were established at random in the area to the east of a 20-ft band of infected saplings bordering a road, so as to allow the prevailing westerly winds to carry released spores to the traps.

Each trap consisted of two glass microscope slides, 2.5×7.5 cm, lightly coated on one side with vaseline and held vertically or horizontally by metal slots, 2.5×2.5 cm, mounted atop a 5-ft pole. The coated surface of the horizontal slide faced upward; that of the vertical slide westward into the wind. Protection from rain was afforded by a sheet metal plate, 8×8 in., mounted horizontally above the slides (Fig. 3). All stems were removed in a 5-ft radius around each station, thus ensuring that no contact occurred between the slides and sporulating infections on leaves.

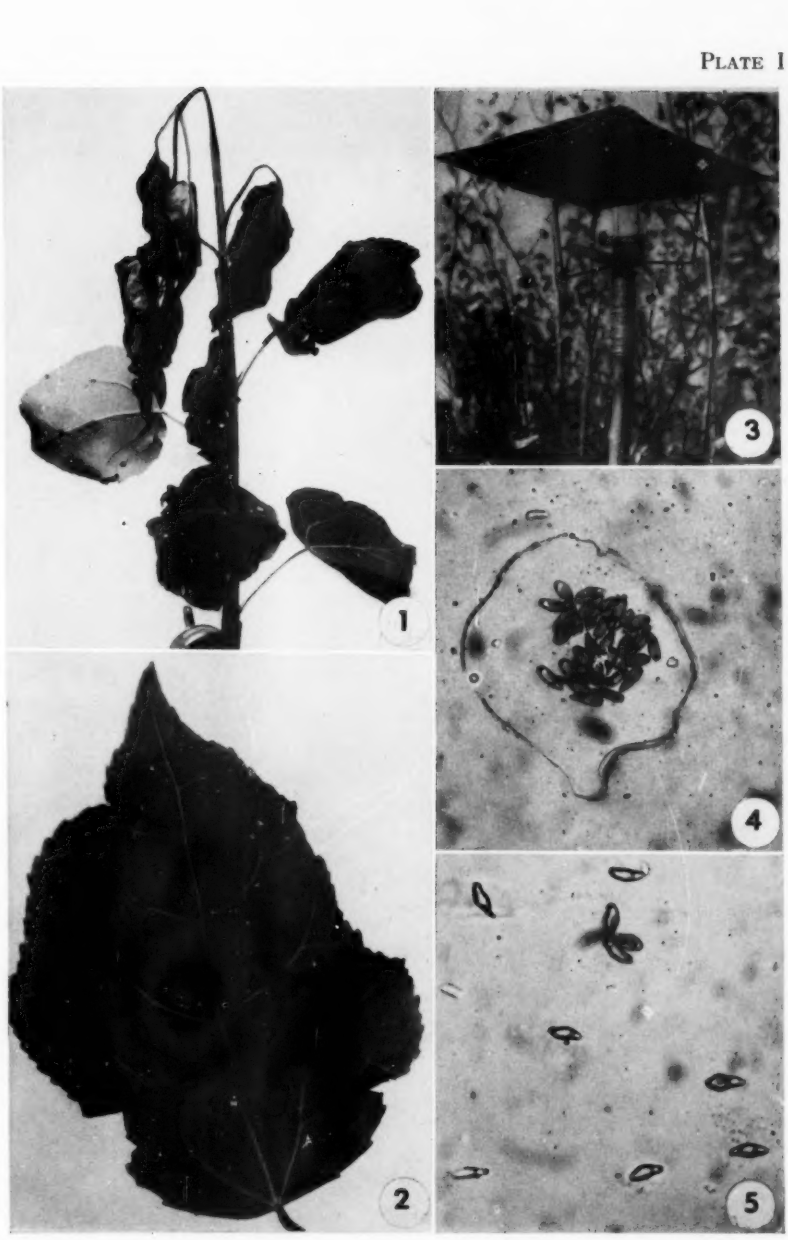
In addition to the trapping stations, two meteorological stations were located in the experimental area. Each contained a thermograph and hygrograph of the portable, 7-day type, housed in a roofed, open-sided shelter set 5 ft above the ground. Since the records of the stations did not vary significantly, they were averaged to form a single set of continuous temperature and relative humidity readings for the observational period.

Other daily weather phenomena were described. Rainfall was classified as absent, light, moderate, or heavy; wind as calm, low, moderate, or high; and sky condition as clear, with sunny intervals, or overcast.

Daily sampling of the spore content of the air commenced on June 6 when sporulating lesions of *P. radiosa* were prevalent, and ended on September 19 when the development of new infections had nearly ceased. At 10 a.m. every day, fresh slides were inserted into the slots in the traps, and the hygrographs and thermographs were inspected to ensure their proper functioning. This routine continued without interruption until sampling ended in September.

The number of spores on each of the eight slides was determined in the

FIGS. 1–5. *Pollaccia radiosa*. Fig. 1. Shoot dieback and leaf blight of trembling aspen regeneration, ×½. Fig. 2. Sporulating lesions on trembling aspen leaf, ×2. Fig. 3. A spore trap used in the study, ×⅛. Fig. 4. A typical aggregation of conidia left by a dried water droplet on a slide. Apparently, surface tension drew the spores together as the water evaporated. The original periphery of the droplet is faintly visible as a thin, discontinuous, white line in the vaseline film. ×340. Fig. 5. Spore deposition pattern obtained on slides on dry days, ×340.



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laboratory with a 10×, or occasionally 43×, microscope objective in conjunction with a 10× ocular. Since conidia of *P. radiosa* are easily recognized beneath the microscope, and because other spores were rarely encountered, counting was a straightforward procedure. The main difficulty was in determining the number of individual spores in aggregations which often occurred about water droplets. The method was to scan the entire exposed surface of each slide, by successively sweeping adjacent parallel strips of the surface each equal in width to the field diameter of the objective, while recording the number of spores with a hand tally counter. The number of spores on each of the eight slides was recorded separately. Then the average numbers of spores on vertical and horizontal slides were determined.

Results

The average monthly numbers of spores trapped on rainy and rainless days during the observational period are compared in Table I according to the position of slides in the traps. These comparisons reveal the existence of several relationships.

TABLE I

Effect of rainfall on the number of spores trapped

Slide position	Month	Average no. of spores collected* per day		Ratio of spores collected on wet vs. dry days
		Wet days†	Dry days	
Horizontal	June	3952	71	
	July	57	7	
	August	365	1	
	September	217	12	
	Average	1472	17	87:1
Vertical	June	872	8	
	July	20	3	
	August	36	1	
	September	7	4	
	Average	310	3.8	82:1

*Based on the totals of four horizontal and four vertical slides, each 7.5 ×2.5 cm, with 12.5 sq. cm of surface exposed, and 6.25 sq. cm enclosed by a supporting metal slot.
†A 24-hour period during which rain showers of any quantity were registered.

I. Effect of Rainfall on Spore Dispersal

Without exception, significantly more spores were trapped on wet days than on dry days by both horizontal and vertical slides. On the average, they collected 87 times and 82 times, respectively, more spores. No relationship was detected between the number of spores collected and the amount of rain that fell during a day. However, prolonged rainfall tended to increase the number of spores trapped; for example, on June 28 and 29 when rainfall was light but continuous, more spores were trapped than at any other time. Hence, it is evident that the dispersal of spores of *P. radiosa* is heavily dependent upon rainfall and its duration.

II. The Relative Efficiency of Horizontal and Vertical Slides in Trapping Spores

A comparison of the average number of spores collected daily by horizontal and vertical slides revealed nearly constant ratios of efficiency between the slides, on both rainy and dry days, in favor of the horizontal slides. The ratios were: (*a*) on wet days, 4.7:1 (1472 vs. 310 spores); (*b*) on dry days, 4.5:1 (17 vs. 3.8 spores). Wider variations were noted when the monthly standard was used; however, horizontal slides invariably collected several times more spores than vertical slides.

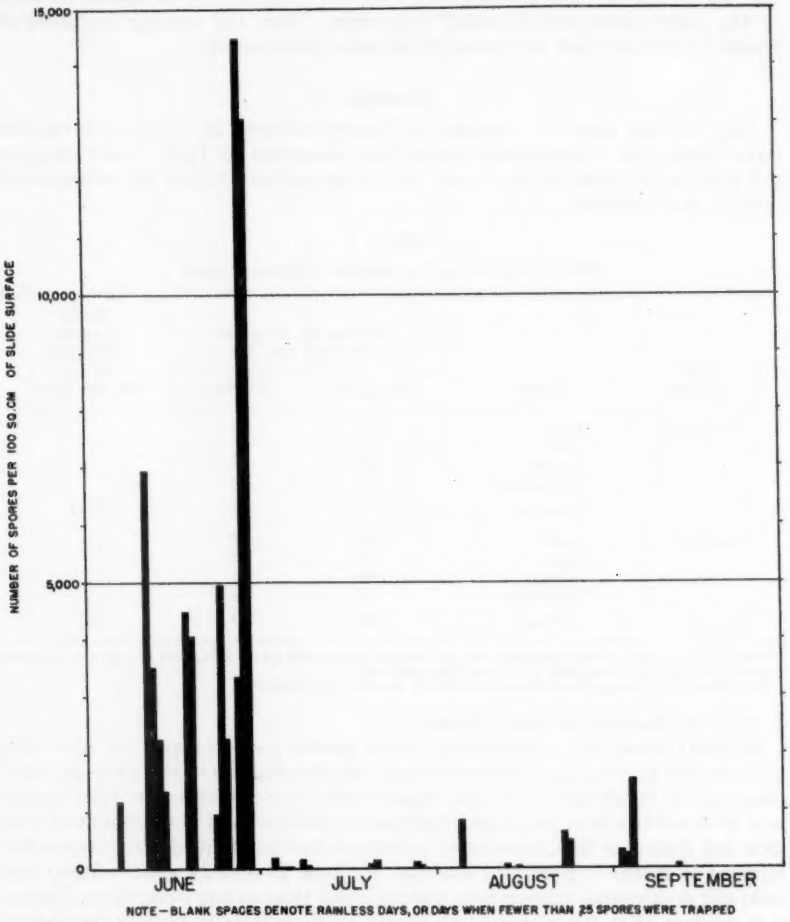

FIG. 6. Number of spores of *P. radiosa* trapped on rainy days, at Chalk River, by four horizontal and four vertical slides; June 6 to September 19, 1957.

No explanation for this greater ability of horizontal slides to trap spores was evident. It may be related to the way in which spores were carried from their points of origin on diseased leaves to the slides or to leaves. This is suggested by the repeated observation that most spores on the slides were, or had been, aggregated about water droplets (Fig. 4). It may be deduced that (1) these droplets result from raindrops striking infected, sporulating leaves; and (2) they serve as spore carriers. Since it is improbable that the force of impact of raindrops on leaves could impel these spore-bearing droplets with sufficient velocity to carry them across the 5-ft gap (the minimum distance) between sporulating leaves and a spore trap, it seems likely that each droplet reaching a slide was assisted in its journey by other agents. Of these, horizontal or near-horizontal air currents at slide level would seem to be the most probable, since the metal roofs above the slides would preclude vertical or near-vertical deposition of spores.

III. Monthly Variations in Spore Content of the Air

Data in Table I show that the average number of spores trapped per day in June was several times higher than the daily average in succeeding months. Furthermore, examination of the daily records for June reveals that most spores were trapped during the latter half of that month with maxima reached on June 28 and 29 (Fig. 6). On each of these continuously rainy days, more spores were trapped by four horizontal slides (12,825 and 10,727 respectively) and by four vertical slides (1626 and 2354 respectively) than the combined totals of similar sets of slides during July, August, and the 19 recorded days of September. The most spores collected on a single slide was 4085 (on a horizontal slide) on June 28.

Since the number of spores trapped at any time probably is proportional to the number of spores in the air, and to the number of actively sporulating infections present, it may be inferred from Fig. 6 that, in 1957, the incidence of infections caused by *Pollaccia radiosa* reached a peak in June and declined thereafter to a much lower and fairly constant level until late September. Although the concentration of spores in the air was not determined from the time that sporulating infections first appeared in May until trapping commenced on June 6, the foregoing inference seems justified because it was observed that sporulating infections were much less prevalent prior to that date than subsequently.

Since the summer of 1957 seemed average in most respects, it appears that the results obtained in this study are indicative of the usual pattern of infection development and spore dispersal in *P. radiosa*.

IV. The Influence of Environmental Factors, Other Than Rain, on Spore Dispersal

The seasonal records were inspected for the existence of correlations between spore dispersal and wind, sky condition, temperature, and relative humidity.

(*a*) *Wind.*—In locating the experimental area and designing the spore traps, it was expected that wind would be one agent assisting spore dispersal in *P. radiosa*. This was the case, but not entirely in the sense anticipated.

Whereas it had been assumed that air currents would carry individual spores to the slides, in actuality, most spores seemed to be moved to the slides in aggregations in airborne water droplets.

(*b*) *Sky condition.*—The only sky conditions found to favor spore dispersal were those accompanying rainfall and discussed previously.

(*c*) *Temperature and relative humidity.*—The average maximum, minimum, and mean daily temperature and relative humidity for the period of investigation are presented in Table II. No correlation was discerned between these figures and the fluctuating incidence of *P. radiosa* spores illustrated in Fig. 6.

TABLE II

Variations in temperature and relative humidity at the experimental site June 6 to September 19, 1957

	June 6–30	July	August	Sept. 1–19
Average daily temperature:				
Max.	80.2	83.7	81.0	74.8
Min.	49.4	50.3	44.4	44.3
Mean	64.8	67.0	62.7	59.5
Average daily relative humidity:				
Max.	98.4	99.6	100.0	100.0
Min.	39.4	35.7	28.0	48.2
Mean	68.9	67.6	64.0	74.1

Discussion

It has been noted that the incidence of spores of *Pollaccia radiosa* in the air on rainy days, as revealed by sampling, reached a peak in late June, and then abruptly declined to a low level where it remained with minor fluctuations until the end of the field season. It is suspected that the growth habit of aspens is responsible for this pattern of spore dispersal.

The author has observed at Chalk River that the formation of new shoots and leaves usually begins about May 8 in trembling aspen, and about May 22 in large-toothed aspen. In both species there is then rapid and continuous formation of these structures until mid-June when this growth slackens, then becoming limited to adventitious shoots and suckers.

It has been established that aspen leaves are susceptile to *P. radiosa* only when they are young (2). Therefore, the incidence of infections at any given time probably is proportional to the prevalence of young shoots and leaves at that time. Similarly, because the incidence of spores in the air probably is proportional to the prevalence of sporulating infections at any given time, it may also be concluded that it is proportional to the prevalence of young shoots and leaves of aspen. Consequently, the sharp decline that occurred in the incidence of spores in the air in late June may be attributed to the decline in the rate of new shoot and leaf formation that took place in mid-June. The continuing high incidence of spores in the air until late June is attributed to the 12-day or longer infection period of *P. radiosa* (2).

Considering the large number of spores collected on slides during wet 24-hour intervals, it is likely that during the growing season few leaves at, and probably below, the 5-ft level in an infected area like that in this study

escape contact with *P. radiosa*. Observations indicate this to be so. It is concluded that leaves which escaped inoculation, as evidenced by an absence of infections, did so because they attained immunity early in the season before primary and secondary infections had raised the incidence of spores in the air to high levels.

While this study has been primarily concerned with *P. radiosa* on trembling aspen, the pathogen was also observed on large-toothed aspen regeneration in the vicinity of the experimental area. However, fewer infections occurred on large-toothed aspen than on trembling aspen. It is believed this difference is due to the later date of shoot and leaf development in large-toothed aspen. This leaves less time for reinfections to multiply and release spores before the rate of shoot and leaf development diminishes in mid-June. When it is considered that the period in which reinfections can multiply in trembling aspen is only about 6 weeks, the significance of the shorter period in large-toothed aspen can be appreciated.

The results of this study suggest that *P. radiosa* can be controlled in aspen plantations if fungicides are applied periodically from the time that leaves emerge from winter buds until mid-June, or if certain silvicultural traits can be incorporated into new hybrids by tree breeders. Characteristics which are likely to minimize the number of infections are late leaf emergence and fast-maturing leaves.

Summary

A study was made at Chalk River, in 1957, of the dissemination of spores of *Pollaccia radiosa* and of environmental and other factors suspected of influencing that activity. Possible methods of controlling *P. radiosa* were suggested. From the results it was established that: (1) spore dispersal is mainly dependent upon rainfall, duration being more important than the quantity; (2) the concentration of spores in the air probably is highest in late June, but thereafter falls to, and remains at, a low level; (3) horizontal slides were several times more effective than vertical slides in trapping spores; and (4) wind, temperature, relative humidity, and sky condition have no perceptible effect on spore dispersal.

Acknowledgments

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ON THE SPREAD OF FOMES ANNOSUS (FR.) CKE.[1]

ERIK JORGENSEN[2]

Abstract

Basidiospores of *Fomes annosus* (Fr.) Cke. were found to be wind-dispersed and to be deposited on soil, seedlings, tools, and other objects in the fields of the Provincial Forest Tree Nursery at St. Williams, Ontario.

Spores deposited on the root systems and needles of red pine (*Pinus resinosa* Ait.) nursery seedlings remained viable and ungerminated for 8 weeks, while the seedlings were kept in shipping bales and storage. Direct infection of seedlings was not observed under these conditions. However, roots of recently cut trees were successfully infected with *F. annosus*, when basidiospores carried on seedlings were transferred to the surfaces of roots wounded in a planting process.

The danger of the spread of *Fomes* root rot from southern Ontario into northern Ontario is discussed. A delay of 2 to 3 years in replanting cut-over areas is suggested as a possible preventive measure against the introduction of *F. annosus* with planting stock.

Introduction

For almost 100 years the spread and establishment of *Fomes annosus* (Fr.) Cke. has been investigated and discussed. The past 10 years have brought great advances in our factual knowledge on this subject. Rishbeth (1950, 1951*a*, and 1951*b*) was the first to demonstrate aerial infection of freshly cut stumps and showed through the treatment of stump surfaces immediately after felling that tree killing was clearly related to the proportion of stumps infected with *F. annosus* (Rishbeth 1952 and 1957).

Jorgensen (1955) suggested that *F. annosus* was spread to new areas with infected fence posts used in proximity to trees in shelter belts. This method of spread has later been demonstrated in a controlled experiment (unpublished data).

Transfer of spores through soil with rainwater was demonstrated by Molin (1957), and Jorgensen (1959) suggested long-distance spread through spores being carried on seedlings and clothes and thence transferred to wounded roots of freshly cut stumps. Further, the possibility of insect transmission of *F. annosus* was mentioned.

Fomes annosus was first recorded from Ontario in 1955 (Jorgensen 1956) and has since been found to occur in only three counties in southern Ontario with the most severely infected area in the vicinity of the Provincial Forest Tree Nursery at St. Williams, Ontario.

Jorgensen (1960) postulated, on the basis of the results of Canadian decay studies and his own investigations, that *F. annosus* had been introduced to eastern Canada through the importation of tree seedlings from Europe in the early years of reforestation some 50 years ago, and also that the fungus at the present time was not found in the natural forests of northern Ontario.

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Reforestation of cut-over areas in northern Ontario was first initiated during the past 5 years, the planting being partly carried out with seedling stock produced in southern Ontario. The threat, inherent in this planting program, of introducing *F. annosus* into northern Ontario's enormous stands of jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* (Mill.) B.S.P.) prompted an investigation of the spread of *F. annosus* in Ontario, which is reported herewith.

The Dispersal and Viability of Air-borne Spores at the Provincial Forest Nursery, St. Williams, Ontario

Methods

The identification of air-borne basidiospores of *F. annosus* is made extremely difficult by their small size (5.5×4.1 μ), inconspicuous color, and indeterminate shape, which exclude the use of spore traps unless the collected spores are cultured. However, this problem was readily overcome by the application of a method first developed by Rishbeth and Meredith (1957). Aseptically cut 1-cm-thick slices of living Scots pine (*Pinus sylvestris* L.) stems approximately 8 cm in diameter were placed on three pieces of filter paper in sterile Petri dishes (9×2 cm). The filter paper was saturated with sterile water, but excess water was not allowed in the dishes. Petri dishes prepared in this manner were used for the collection of spores from the air, the soil, and the surface of needles, roots, and other objects.

Disks of this sort were examined for the presence of *F. annosus* after incubation at 22° C for a period of from 1 to 3 weeks. The characteristic conidiophores of the fungus could readily be identified under a dissecting microscope. However, periodic checks on the identification were made during the study by the production of pure cultures of *F. annosus*, through transfer of conidia to malt agar slants. Direct microscopic examination of the conidiophores with a compound microscope was difficult and time-consuming, because they adhered so tenaciously to the wood slices, whereas they can be transferred more readily to a slide from an agar surface.

Results

Isolation of F. annosus Spores from the Air, the Soil, and the Surface of Needles, Roots, and Other Objects

Ten stations were established in the fields of the Provincial Forest Tree Nursery at St. Williams, Ontario (Fig. 1). At these stations spores were collected from the air during three 24-hour periods by exposing at soil level wood disks in two Petri dishes for 12 successive 2-hour periods. Samples from the soil surface were taken at all stations by lightly touching the soil with six wood disks in each experiment and rapidly replacing them in their dishes. Ten dishes with unexposed wood disks were placed at each station in each experiment and served as checks. Table I gives the results for each station, summarized from the three experiments, which were carried out in August 1960 from noon of the 10th to noon of the 11th, noon of the 13th to noon of the 14th, and noon of the 23rd to noon of the 24th.

Fomes annosus was obtained from all stations in approximately equal amounts except for station No. 2, where the fungus was found on only two occasions.

FIG. 1. Map of the Provincial Forest Station at St. Williams, Ontario, showing the location of the 10 sampling stations for spores of *Fomes annosus*.

TABLE I

Isolations of *Fomes annosus* from 10 stations at the Forest Nursery at St. Williams, Ontario

Medium	Exposure time*	Number of wood disks with *Fomes annosus* at station No.: 1	2	3	4	5	6	7	8	9	10	Disks: With fungus	Disks: Total no.
Air	0–2	4	0	4	4	2	4	3	0	2	4	27	60
	2–4	5	1	5	4	4	4	2	6	4	4	39	60
	4–6	5	0	3	5	3	6	3	4	2	2	33	60
	6–8	4	0	2	3	2	4	2	3	1	2	23	60
	8–10	0	0	0	2	1	4	0	2	0	1	10	60
	10–12	1	0	0	0	0	2	0	1	0	2	6	60
	12–14	0	0	0	0	0	0	0	0	0	0	0	60
	14–16	0	0	0	0	0	0	0	0	0	0	0	60
	16–18	0	0	0	0	0	0	0	0	0	0	0	60
	18–20	2	0	2	0	2	4	2	1	3	4	20	60
	20–22	4	1	4	2	4	6	2	2	0	4	29	60
	22–24	6	0	5	3	4	6	4	0	4	2	34	60
	Checks	0	0	0	0	0	0	0	0	0	0	0	300
	Total	31	2	25	23	22	40	18	19	16	25	221	1020
Soil	Noon	13	0	0	0	0	0	0	0	0	0	13	180

*Successive 2-hour exposures starting at midnight.

Station No. 1 was in a severely attacked, 50-year-old red pine stand, station No. 2 was situated under the canopy of trees in the border of a transplant field, while the remaining stations all were in open fields. Its position may thus explain the findings for station No. 2.

The experimental periods were without rain and characterized by warm sunny days and dark clear nights. This may explain the lack of isolations in the afternoon hours, found to be constant in all three time periods, and believed to be due to rising air currents caused by the soil heat at this time of day.

Very little rain fell in the later part of July and the beginning of August. Preceding the first two experimental periods light rain fell on August 3rd (0.25 in.) and on August 5th (0.08 in.). The total number of infected disks which were obtained was very similar in these two trials, namely 56 and 61 out of 240 disks exposed in each experiment.

On August 15th, 0.77 in. of rain was recorded and rain fell again on August 22 (0.12 in.), which may account for the fact that 119 disks out of 240 exposed were infected in the third experimental period.

A search for fruiting bodies of *F. annosus* in known severely attacked stands in the area revealed only a few active pore layers above the litter layer, and for this reason it was feared for awhile that the experiments would be unsuccessful. Therefore, it is to be expected that the recorded figures are small compared with those that would be obtained in the same area in September/October, and November, when active fruiting bodies occur abundantly on stumps and on diseased trees.

From the results it is estimated that the number of spores settling from the air averaged 200,000 per acre in a 24-hour period. This figure was estimated by assuming that each infected wood disk was infected by the germination of only one spore, which was not always the case as evidenced by the observation of widely separated colonies of *F. annosus* on a number of the infected disks.

The soil isolations were unsuccessful except at station No. 1, where the samples were obtained by contact with the litter layer, while in all other stations sampling was attempted from cultivated soils free of litter. The reason for failure in the soil surface isolation in general is not clear, and was not further investigated.

In addition to these experiments, isolation of *F. annosus* spores was obtained by touching the surface of a number of objects found in the nursery fields with sample disks, that were rapidly replaced in the Petri dishes. Table II lists the objects and the number of trials. Five-minute exposures of check disks were made in proximity to the investigated objects and it must be concluded from the results that spores accumulated on the surface of the objects investigated. These observations are similar in nature to the findings of Rishbeth and Meredith (1957).

The Viability of Air-borne Spores

It was considered that for this investigation the viability of air-borne spores settled on nursery seedlings was of prime interest. Therefore, fruiting bodies of *F. annosus* were collected and placed on bundles of 2/2 red pine

TABLE II

Isolation of *Fomes annosus* from the surface of different objects in the nursery fields at St. Williams, Ontario

Objects sampled	Number of exposed wood disks	
	With fungus	Total
Nursery seedlings (tops)	8	18
Tools (tractor, cultivator, etc.)	2	16
Investigator's working clothes	6	14
Investigator's hair	2	12
Total	18	60
Check (5 minutes' exposure of disks at sample place and time)	3	50

seedlings. Wet cotton was placed on top of the fruiting bodies and a moist chamber was provided by covering with overturned pails (Fig. 2) for a 24-hour period, a procedure which assured a very heavy coating of the seedlings with basidiospores. The treated bundles of seedlings, after removal of the pails, were packaged in normal burlap shipping bales. Isolations were then attempted from the bundles at weekly intervals using wood disks as previously described.

Several experiments were carried out unsuccessfully until the fall of 1960, when it was found that viable spores could be obtained from stored bales 8 weeks after the seedlings had been inoculated with spores. This experiment was somewhat different from the previous unsuccessful ones. The seedlings were lifted late (October 6, 1960), and each bundle was wrapped in plastic material and not watered before being packaged in the shipping bale. The bale was transported to Toronto from St. Williams during the first week of the experiment. Here it was opened and after removal of the plastic the seedling bundles were placed in storage outside the laboratory in a wooden box, which sheltered them from sun and rain.

The weekly isolations were carried out using 20 wood disks, 10 of which were kept as checks. For the first 7 weeks all 10 exposed disks were heavily infected with *F. annosus*. In the 8th week, 8 out of 10 isolation attempts were successfully accomplished.

The seedlings were in good health at the end of the experiment and, upon examination, were found to be uninfected by *F. annosus*. However, a large number of ungerminated spores were present on needles and roots.

The previous unsuccessful experiments are attributed to excessive watering and the influence of higher temperatures, associated possibly with interference from the microflora of sphagnum, which was not used in the last experiment.

Basidiospore Infection of the Root Systems of Young Seedlings and Freshly Cut Larger Trees

Spore infection of living roots of red pine seedlings was attempted by covering the root systems of bundled seedlings with basidiospores as described above. Four hundred seedlings were used in the experiment; half of

PLATE I

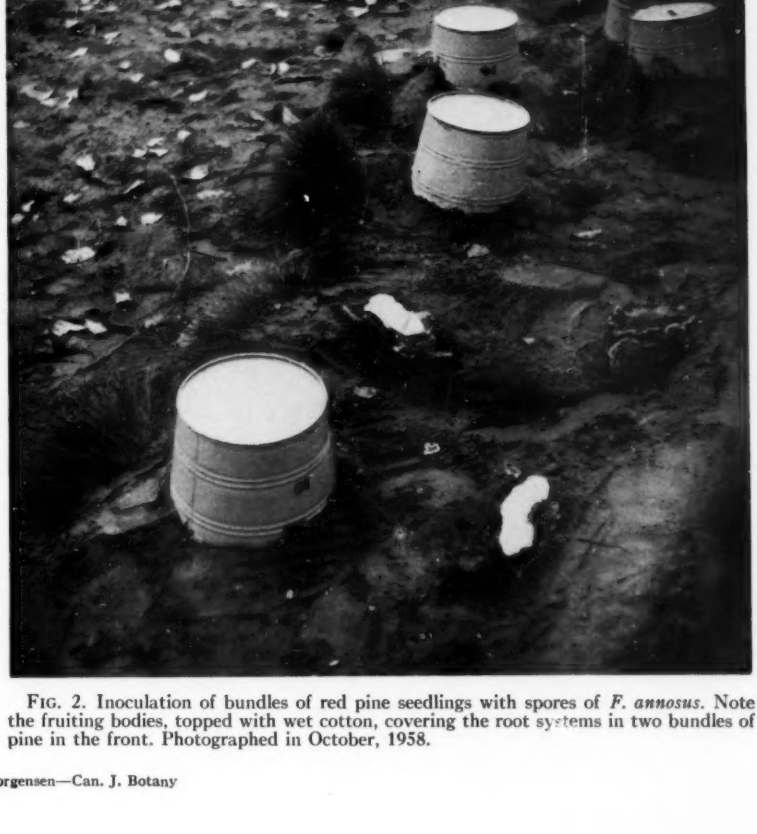

FIG. 2. Inoculation of bundles of red pine seedlings with spores of *F. annosus*. Note the fruiting bodies, topped with wet cotton, covering the root systems in two bundles of pine in the front. Photographed in October, 1958.

them, or eight bundles, were wounded severely on the roots by being slapped against a wooden board before the inoculation. The other half were not artificially wounded but had small wounds as a result of the lifting of the seedlings from the transplant beds. After inoculation the seedlings were planted in a nursery field in four rows of 100 seedlings.

The plot was kept under observation for 2 years, during which no seedlings showed any disease symptoms. The seedlings were then lifted and an examination of the root systems revealed that dead roots or rootlets were not present and that all seedlings, in spite of the treatment, had remained healthy.

Spore infection of living roots of freshly cut trees of different ages was attempted by direct transfer of basidiospores to wounds on roots of trees 14 days after felling. Large fruiting bodies of *F. annosus* were used for the collection of spores on sterile paper in glass jars, and the spores were transferred with a brush to wounds on roots remaining *in situ*. Proper checks were provided by uninoculated wounded roots on the same stumps.

TABLE III

Results of spore inoculation of wounded roots

Tree species	Age	No. of trees	No. of roots		
			Inoculated	Infected	Checks
Pinus banksiana Lamb.	9	4	7	4	8
	18	5	14	8	10
Pinus resinosa Ait	14	3	8	5	9
	18	4	12	7	11
Pinus sylvestris L.	8	4	15	4	13
	20	2	6	3	4
Pinus strobus L.	16	4	8	6	7
	21	2	7	3	4
	Total		77	40	66

Table III gives the results of this investigation, evaluated 8 months after inoculation, by removal of the treated roots to the laboratory where they were split, incubated for 6 days, and examined for the presence of conidia. The results are in agreement with those published by Wallis (1961).

The wounds on the stump roots were made with a spade simulating a planting operation and care was taken that larger roots 5–6 cm in diameter, as well as small roots up to 1 cm in diameter at the point of wounding, were well represented in the experiments. However, no difference in the occurrence of infections in relation to root diameter was observed.

Discussion

The results of these experiments have clearly established the risk of long-distance spread of *F. annosus* through planting operations or research activities on recently cut-over areas.

Stump infection through air-borne spores was well established by Rishbeth (1950 and 1951). However, there is no evidence at the present time

that this type of spread has taken place over long distances in Ontario (Jorgensen 1960).

An explanation for this apparent lack of long-distance spread may be found in a paper by Yde-Andersen (1961), who found that in Denmark stump infections take place only when certain temperature and precipitation conditions are met before and at the time of cutting. In September, when *F. annosus* fruiting bodies occur abundantly in southern Ontario, fall conditions are, in most years, well advanced in northern Ontario.

The reforestation of cut-over areas initiated in recent years in northern Ontario could lead to the establishment of *F. annosus* in this area. If this happened, there is no reason to believe that the fungus would not spread by air-borne spores from such a center. The occurrence of *F. annosus* in Alaska and in northern Scandinavia suggests that the climate of northern Ontario would not prohibit the spread of the fungus. It must therefore be anticipated that an introduction of *F. annosus* into northern Ontario would prove most serious to the northern forests.

Rishbeth (1950, 1951, and 1957) has developed a stump surface treatment for the control of stump infections. However, this control method appears at the present time to be unsuitable for the conditions in northern Ontario. *F. annosus* has been present at St. Williams, Ontario, for at least 30 years and has in that time shown only local spread in southern Ontario, a condition which would not justify a treatment of the enormous number of stumps made annually in northern Ontario at the present time. However, protective measures against the introduction of *F. annosus* with nursery seedlings might be necessary.

Rishbeth (1950 and 1951) and Meredith (1959) and others have investigated the competition between *F. annosus* and other microorganisms on roots and stumps. Their conclusion is that stump surfaces are invaded with a flora, which will limit the chances of *F. annosus* infection if this fungus is absent at the time of cutting and shortly thereafter. Components of this flora adverse to *F. annosus* will later spread to the stump roots. It is therefore believed that a delay in planting of cut-over areas of from 2 to 3 years will considerably reduce, if not exclude, the introduction of *F. annosus* to cut-over areas with seedling stock.

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SEROLOGICAL AND PHYSICAL PROPERTIES OF SOME STONE-FRUIT VIRUSES: NON-VIRUS PARTICLES ASSOCIATED WITH INFECTION[1]

R. S. Willison, J. H. Tremaine, and M. Weintraub[2]

Abstract

Cherry yellow virus, isolates Y.2 and Y.4, and necrotic ring spot virus, isolates N.4 and N.5, purified either from cucumber leaves or from sour cherry petals, were characterized by antigenically related particles that sedimented at approximately 72 *S*. Some preparations of each virus derived from either host also contained either a 35 *S* or a 22 *S* component usually having a low nucleic acid content. Such preparations were shown by the agar gel double-diffusion technique to contain two antigens, V and Q, that were only distantly, if at all, related. The 72 *S* component is associated with the V antigen, whereas the 22 *S* and 35 *S* components are tentatively considered to be two forms of the Q antigen. The Q antigen could also be detected in clarified expressed sap of infected cucumber tissue, but not in that of healthy cucumber nor in extracts prepared from healthy sources by methods used to purify the viruses. The Q antigen is thus associated with infection, but its origin has not yet been satisfactorily determined.

Introduction

The association of more than one type of particle with infection has been reported for several 'spherical' plant viruses. The best-known example is the occurrence in turnip yellow mosaic virus preparations of several types of protein and nucleoprotein particles similar in size, but different in density and biological activity (4, 5). A similar phenomenon has been described for the viruses of squash mosaic (7) and wild cucumber mosaic (8). The several components associated with each of these viruses do not appear to be artifacts resulting from purification procedures and probably occur *in vivo*. On the other hand, in purified preparations of turnip yellow mosaic virus, the virus component has been shown to break down with the release of nucleic acid either in alkaline solutions (2) or between pH 6.0 and pH 8.0 after short periods of mild heating at temperatures well below the thermal inactivation point (3). The products of thermal degradation differed in physical characteristics from those of alkaline degradation.

Previous reports from this laboratory (12, 13, 14) have intimated that some members of the cherry-yellows group of stone-fruit viruses may be inactivated or even disintegrated more easily than others during purification by procedures that have little or no apparent effect on other more stable viruses. The present paper is an initial report of non-virus particles found in association with cherry yellows and necrotic ring spot viruses in infected tissue.

[1]Manuscript received June 1, 1961.
Contribution No. 17, Research Laboratory, Research Branch, Canada Department of Agriculture, Vineland Station, Ontario.
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Materials and Methods

The isolates used in this phase of the investigation were N.4 and N.5 of the necrotic ring spot virus and Y.2 and Y.4 of the cherry yellows virus, purified either from sour cherry petals, MP, as previously described (12), or from cucumber cotyledons and leaves by method DS_5, which consists of clarification by dialysis against acetate buffer at pH 4.6 and two cycles of differential centrifugation (13). Samples of expressed sap from healthy cucumber leaves ($H.S_1$) and from infected cucumber leaves ($N.5.S_1$), clarified by centrifugation for 15 minutes at 10,000 *g* (max.), were also used in some serological tests. Purified virus preparations are identified in this paper by a code indicating the isolate, the source or method of preparation, and the year. For example, N.5.MP.60 refers to a preparation of isolate N.5 purified from Montmorency cherry petals in 1960 and $Y.2.DS_5.58$ to one of isolate Y.2 purified from cucumber by method DS_5 in 1958. Purified preparations were stored in a refrigerator at 4° C until used.

Rabbit antiserum was prepared by intramuscular injection of an oil emulsion of antigen preparation into the thigh (9, 13). The code identifying an antiserum is that of the antigen extract used in its preparation prefixed by "anti-", e.g. anti-N.5.MP.58. Precipitation tests were made by agar gel diffusion in 60-mm Petri dishes. Wells of approximately 0.15-ml capacity were cut in the agar 8 mm apart, edge to edge. When precipitation was developed the distance of the precipitin zone from the antigen well was measured to the nearest millimeter (9).

The content in mg per ml of nucleic acid (RNA) and protein in each preparation was estimated from the optical densities at wavelengths of 260 and 280 mμ by means of a nomograph based on the data of Warburg and Christian (10). With highly purified virus preparations, calculations of RNA and protein content based on the same criteria have given results that satisfactorily agreed with those obtained by chemical analyses (6). The percentages of RNA given in this paper are characteristic of the preparations rather than of the virus entities, since the proportion of virus to other components varies somewhat from preparation to preparation. Yields are given as mg nucleoprotein per 100 g starting material.

Sedimentation rates (*S*) were calculated from data obtained with a Model E Spinco ultracentrifuge, usually at 31,410 r.p.m. The *S* values were corrected for viscosity.

Results and Discussion

Physicochemical Characteristics

According to analytical centrifuge data, the components detected in the purified preparations fell into four groups that sedimented in the ranges: 66 to 75 *S*; 35 to 44 *S*; 22 to 23 *S*; and 5 to 7 *S* (Table I). For convenient reference, these groups are designated in this paper as the 72 *S*, 35 *S*, 22 *S*, and 6 *S* components respectively.

Since infective preparations were found to contain the 72 *S* component, either alone or accompanied by one or other of the 22 *S* or 35 *S* components, the 72 *S* component is evidently closely associated with the virus. It is tentatively considered to be the intact virus but, because its sedimentation rate is

low in relation to the reported size of the virus particles (12), it may well be a 'ghost' of the virus that has lost part of its original complement of RNA. The 22 *S* and 35 *S* components have been found only in virus preparations, but apparently did not occur together (Table I). These components were associated with a depression of yield and RNA content in the MP preparations, except N.5.MP.58, and with a low RNA content in the DS_5 preparations (Table I). Since the 72 *S* component contained approximately 9% RNA (Table I), the 35 *S* and 22 *S* components were evidently proteins or non-virus nucleoproteins with low nucleic acid contents. The 6 *S* particles, detectable in some preparations, were presumably free nucleic acid or aggregated nucleotides.

TABLE I

Sedimentation rates of components and nucleic acid (RNA) content and yield of nucleoproteins in purified preparations of isolates N.4, N.5, Y.2, and Y.4

Preparation	Sedimentation rates (*S*)	RNA, %	Yield, mg/100 g*
N.5.MP.58†	6, *35*,‡ 75	8.0	31.0
N.5.MP.60	22, 73§	5.5	16.4
N.5.DS$_5$.59	44?, *72*, 102, 109	7.2	2.07
N.4.DS$_5$.60	75	5.8	1.95
Y.2.MP.58a	6, *69*	8.6	32.0
Y.2.MP.58b	5, 38, *70*	6.4	17.3
Y.2.MP.60	7, 23, 66§	6.2	17.0
Y.4.DS$_5$.58a	73	9.4	0.4
Y.4.DS$_5$.58b	22, 74§	4.1	0.9

*mg nucleoprotein per 100 g starting material.
†Preparations in italics were used for preparing antisera.
‡S value in italics is that of the predominant component.
§Components present at similar concentrations.

Preparations N.5.MP.58 and Y.2.MP.58a were purified simultaneously from cherry petals collected the same morning. The analytical centrifuge runs were made 2 weeks later. The 35 *S* component occurred at relatively high concentration in the N.5 preparation, but not in the Y.2 (Table I). The yields and RNA contents of the two preparations were comparable. However, the 35 *S* component did appear, associated with a loss of RNA and a lower yield, in Y.2.MP.58b prepared from petals collected from the same trees 4 days later. These observations suggested that this non-virus component may be dissociated or disintegrated virus, accumulating in the Y.2 infected petals as they aged and in the N.5 preparation but not the Y.2 *in vitro* during refrigerated storage in accordance with the view that Y.2 is the more stable *in vitro*. In 1960, wet weather delayed petal harvesting until late in the bloom period, when the petals were long past their prime. The absence of the 35 *S* component and the presence of the 22 *S* in N.5.MP60, Y.2.MP.60, and similar preparations of other isolates appeared to be more than coincidental and suggested that a further disintegration of the virus had occurred *in vivo*. The 22 *S* component was not usually detectable in Y.4 preparations from severely infected cucumbers (e.g. Y.4.DS_5.58a, Table I), but was abundant in Y.4.DS_5.58b purified from cucumber plants showing only mild symptoms.

The N.4.DS_5.60, which had a relatively low RNA content, contained some normal host contaminant, detected serologically, but no appreciable amount of 22 *S* or 35 *S* component.

Serological Relationships

Representative examples of precipitin reactions between antigen preparations from different sources and antisera that could be expected to contain antibodies against 22 *S*, 35 *S*, and 72 *S* components are presented in Table II. It has already been shown (9) that the necrotic-ring-spot and cherry-yellows isolates have antigenic properties in common. The virus (V antigen) precipitated approximately 3 mm from the antigen well (N.4.DS_5.60 vs. anti-Y.4.DS_5.58b, Table II). A second component of the Y.2.MP.60 preparation must therefore have been responsible for the formation of the zones in the 5-mm region (Table II). This component, which has been tentatively designated as the Q antigen, was also present in clarified expressed sap from infected cucumber, N.5.S_1, but not in the clarified sap from healthy cucumber, H.S_1 (Table II). Anti-N.5.MP.58 and anti-Y.4.DS_5.58b were also tested against extracts of healthy cucumber leaves and cotyledons and of healthy cherry petals, prepared by several different procedures used for purifying the viruses. They contained no antibodies reacting with normal host antigens. The Q antigen is therefore associated with infection in both cucumber leaves and cherry petals. It also occurs in preparations of other isolates of the cherry-yellows group (9) purified by the method described by Fulton (1).

TABLE II

Differential serological precipitation of components of virus preparations in agar gel diffusion plates

Antisera and components	Antigen preparations				
	N.4DS_5.60	Y2.MP.60			
	(72 *S*)	(22 *S*)	(72 *S*)	N.5.S_1*	H.S_1*
Anti-N.5.MP.58 (35 S)	—†	5‡		5	0†
(72 *S*)	—		3	3	0
Anti-Y.4.DS_5.58b (22 *S*)	0	5		5	0
(72 *S*)	3		3	3	0

*Clarified expressed sap of infected (N.5.S_1) and healthy (H.S_1) cucumber leaves and cotyledons.
†0, no precipitin zone; —, not tested.
‡Distance of precipitin zone from edge of antigen well, measured to the nearest mm.

The Q antigen evidently diffused farther than the V antigen before meeting its antibody in optimal proportion. Since diffusion rates vary inversely with particle size and directly with concentration, the position of the Q-precipitin zone could have been due to the Q antigen being smaller than the V antigen or greatly in excess of its own antibody or both. In the first event, the Q antigen would presumably correspond to the 22 *S* component in the Y.2.MP.60 preparation. That the Q antigen was precipitated by both anti-N.5.MP.58 and anti-Y.4.DS_5.58b suggested that the 35 *S* component in the former was another form of Q antigen, unless of course the Q and V antigens are

closely related. In any case, an alternative hypothesis would require the improbable postulates that the proteinaceous 22 *S* and 35 *S* components were non-antigenic and that the Q antigen, if different from V, was not detectable in the analytical centrifuge.

To determine the degree of relationship between the Q and V antigens, preparation N.5.S_1 was titrated in December, 1959, in a series of twofold dilutions against anti-N.5.DS_5.59, and vice versa. According to the antigen titration, Q antigen was detectable up to a dilution of 1:16 and the V antigen only up to a dilution of 1:4, so that the Q/V ratio was at least 4:1 in N.5.S_1. On the other hand, titration of the antiserum indicated the titer of the Q antibody to be 1:2 and that of the V antibody to be greater than 1:32. Thus the Q antibody/V antibody ratio in anti-N.5.DS_5.59 was less than 1:16 and the V antibody did not react to any appreciable extent with Q antigen. Consequently Q antigen can at best be only distantly, if at all, related to V antigen and the position of the Q-precipitin zone may sometimes be due to antigen excess, at least in part.

The unrelatedness of the two antigens poses a dilemma regarding the origin of Q. If the 22 *S* and 35 *S* components are forms of Q antigen and, as suggested earlier, are products of virus disintegration, either the surface of the intact virus must have been profoundly modified in the process or two viruses were involved, one of which was relatively stable and the other labile and rarely present in intact form *in vitro*. If two viruses were present and intact in the preparations, they were apparently similar in size and sedimentation rate and their precipitin zones were superimposed. Such coincidences are possible, since, for example, the unrelated peach yellow bud virus fulfills requirements of size and sedimentation rate when purified from cowpea and tends to be less stable in cucumber than in cowpea extracts (11). Alternatively, the Q antigen may have been a by-product of the abnormal metabolism of the infected cells. If so, it is remarkable that the same antigen should occur both in cherry petals and in cucumber leaves and cotyledons. These aspects of the problem are being investigated.

Acknowledgments

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TYPIFICATION AND SYNONYMY OF PENIOPHORA SPECIES SECT. TUBULIFERAE (CORTICIACEAE)[1]

LUELLA K. WERESUB

Abstract

As a preliminary to discussion on the specific limits and affinities of species of *Peniophora* sect. Tubuliferae, a report is made of the taxa known to have been treated in connection with this section or as members of it, and descriptions of type or designated lectotype material are given wherever possible. *Nomina confusa*, misapplied names and names that, from the type descriptions given, appear to be obvious facultative synonyms, are pointed out as necessary discards.

Introduction

By erecting the genus *Tubulicrinis*, Donk (1956*b*) has supplied a much-needed niche for a selected number of species previously grouped mainly in Bourdot and Galzin's ([1928]) subsection * * *, section Tubuliferae of that most heterogeneous of genera *Peniophora* Cke s.l. And Eriksson (1958) has introduced members of subsection * * * * into his new genus *Hyphodontia*. Other genera are already available, or will probably soon be provided, for Tubuliferae species which fit into neither of these new genera, and certainly not into the genus *Peniophora* s.s. (cf. Eriksson 1950, *et al.*).

This seems to be an appropriate time to present a study of the section Tubuliferae in detail, to reconsider the species individually before consigning them to positions among their closest relatives. At this point in our study, therefore, the new generic groups will be used primarily to avoid the complete heterogeneity of an alphabetical treatment rather than to affirm the relationships indicated. Attention is paid to all species that I know to have been included in the Tubuliferae by various authors, and to some of the reported infraspecific taxa of which authentic material was available or which seemed to me to require present-day reference.

Since the types of some of the species concerned have not been fixed as yet, the protection afforded by the International Code of Botanical Nomenclature against the usage of a name in different senses has been unavailable (cf. p. 1475 below, discussion under *Odontia abieticola*). It is the purpose of this first paper in this study to try to eliminate at least one source of uncertainty by the selection of lectotypes wherever necessary and possible. Descriptions will be given here, not of the species, but of the specimens which serve as type or lectotype, so that the decisions made at this time regarding facultative synonyms, and the specific circumscriptions to be made in future, may be judged on the basis of the characteristics which pertain to the "epithet-bringing" material. It is hoped, though not promised, that the characters

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that may eventually prove to be of taxonomic significance will have been adequately described herein.

In an attempt to fill this requirement, particular attention is paid to the development of basidia and cystidia. Pleurobasidia (Donk 1956*a*, 1957) have already been assigned what appears to be a justifiable taxonomic status. Cystidia of like developmental origin have been weighed (Donk 1957; Weresub 1957) for their possible value in uncovering relationships. Therefore I strive here to distinguish between basidia and cystidia which are terminal to the hyphae that bear them, and those which are lateral extensions from those hyphae: basally furcate structures with one fork a "dead end" are called "falsely" bi-rooted, and those whose both forks end in clamps in the hypha from which they stem are called "truly" bi-rooted. It remains to be seen if such distinctions can serve to clarify the picture of relationships.

An explanation must be given, also, of the treatment accorded Bourdot and Galzin binomials. To review the situation briefly: in their series of papers on the "Hyménomycètes de France", Bourdot and Galzin (1909–1925) assigned binary names to taxa at subspecific as well as specific levels. In some cases, these names are followed by the notation "nov. sp." or "nov. subsp."; other new names are given no designated rank, but some are presented in "slanted characters", and some in bolder type than others. In their summarizing book, "Les Hyménomycètes de France", Bourdot and Galzin ([1928]) used an expanded set of typographic distinctions, explaining their meaning in the introduction on p. iv, as follows: "Les noms des espèces sont imprimés en caractères gras, ceux des sous-espèces en caractères penchés, ceux des variétés en petits caractères gras et ceux des formes en italiques."

On account of this explanation, as Rogers and Jackson (1943) and Donk (ms., unpublished) point out, there is no doubt about the intended status of each taxon described in the 1928 book. There is also no possible doubt about the taxonomic rank of those binomials in the 1909–1925 series which are explicitly designated as either species or subspecies.

The subspecific binomials have been treated by later authors in several ways. Some have been accorded specific rank as though the original authors had so intended them (*Peniophora accedens* in Wakefield and Pearson ([1919]); *P. abietis* in Bourdot and Maire (1920); *P. subulata* in Donk (1930, 1931); *P. propinqua* in Laurila (1939); *Odontia abieticola* in Lundell and Nannfeldt (1939), and in Christiansen (1953)). In other cases, the obviously subspecific binomials have been simply corrected to the legitimate trinomials (e.g. *P. glebulosa* ssp. *subulata* in Wakefield and Pearson (1918); *O. barba-jovis* ssp. *abieticola* in Pilát (1933); *P. cremea* ssp. *allescheri* in Rogers and Jackson (1943); *O. alutacea* ssp. *floccosa* in Eriksson (1958); etc.). On the other hand, Saccardo *et al.* (1925) and Donk (1956*b*) felt it necessary to use the Bourdot and Galzin infraspecific binary combinations unchanged; while Weresub (1953) adopted the extreme legalistic point of view, interpreting the "not admissible" of the then Art. 34 of the International Code (now Art. 24, cf. Lanjouw *et al.* 1956) as meaning "illegitimate", and so ignored the publication of the infraspecific binomials. However, after discussing the matter with Professor D. P. Rogers, reading Dr. Donk's manuscript (unpublished), and rethinking the problem, I have become convinced that my previous approach

is a barren legalism. Here, therefore, the simpler, more logical, more customary usage is accepted—the correction to the trinomial—because this interpretation, when consistently followed, provides a consistently satisfactory solution.

The question of the undesignated binary combinations in the Bourdot and Galzin 1909–1925 series is a different one. To suggest a retroactive application of the 1928 explanation is to ascribe to Bourdot a superhuman constancy which he did not display in this instance. Both Professor Rogers (*in litt.* 1952) and Dr. Donk (ms., unpublished, 1959) pointed out to me numerous examples of typographic treatment in the earlier papers differing from that in the 1928 book. It seems clear that Bourdot played with several typographic schemes before he settled on the one followed in 1928. And so, the status of the unranked binomials in the earlier series must be decided without the guidance of the later explanation.

Donk (ms., unpublished) argues persuasively for an interpretation that will not violate the authors' intent. To me, it seems that the attempt to make such interpretations may give rise to unnecessary rivalries about the relative values of unconfirmable solutions. The only incontrovertible facts are that the names of the unranked taxa were published as binomials, and that binomials, according to our present rules, denote taxa of specific status alone. Since we have no statement of intent from the authors, the unranked binomials in the 1909–1925 series are here taken as legitimately published specific names, whatever their typography. So much for the nomenclature to be followed.

Microscopic examinations of the dried fruit-bodies were made in the conventional way (Weresub 1953): under oil immersion objectives, squash mounts, either in phloxine with ca. 3% KOH, with or without the addition of ammoniacal Congo red, or in Melzer's reagent as a test for amyloidity.

It is with particular gratitude that I acknowledge my debt to Dr. D. P. Rogers, now professor at the University of Illinois, for without his skillful guidance and irresistible encouragement this work would never have been done; but he is not to be held responsible for my interpretations and decisions, with which he may wholly disagree. My appreciative thanks are extended also to my professor at the University of Toronto, Dr. R. F. Cain, for his interest and ever-generous assistance; to others who have read my thesis and this script and discussed them with me; to Dr. M. A. Donk and Professor Rogers for the liberal loan of specimens from their personal herbaria, as well as the curators of fungal collections in the following herbaria from which critical material was borrowed: the National Fungus Collections in Beltsville (BPI),[2] the Farlow Herbarium at Harvard (FH), the Kew Herbarium (K), the Rijksherbarium at Leiden (L), the New York Botanical Garden (NY), the National Museum of Natural History in Paris (PC), the Natural History Museum at Stockholm (S), the University of Toronto (TRTC), and the Institute of Systematic Botany at Uppsala (UPS). From the New Zealand Plant Diseases Division (P.D.D.), Dr. Cunningham generously sent portions of collections as a gift to TRTC; and from the Scientific Institute in Morocco

[2]Herbarium abbreviations are in accordance with those listed in the Index Herbariorum, Part I, fourth edition. Utrecht 1959. Names of herbaria not listed therein are referred to in full or by their initial letters.

(RAB), Dr. Malençon kindly provided syntypic material of *P. incrassata* for deposit at NY.

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Tubulicrinis Donk 1956*b*

Among other characteristics used in the original diagnosis of this genus is the presence of clavate basidia and of "lyocystidia", i.e. cystidia whose thickened walls are composed of a relatively broad layer, abruptly soluble in 10% KOH [and unstained by ammoniacal Congo red, median to thin or somewhat thickened, insoluble, outer and inner layers stainable by Congo red]. All the Tubuliferae I know to be characterized by such cystidia and basidia are treated here under their pre-*Tubulicrinis* combinations, preparatory to a future consideration of the limits of the genus.

PENIOPHORA GRACILLIMA[3] Ell. & Everh. ex Rogers and Jackson, Farlowia, **1**, 317 (1943)

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN FH, NY
(on rotten maple, Newfield, N.J.; Aug. 1885; J. B. Ellis)

FRUCTIFICATION cream to yellowish, soft cushiony, continuous but with surface minutely granular, almost approaching a subgrandinioid mounding, distinctly hispid with cystidia; separable; margin thinning into indeterminate hispidity. TRAMA well over 80 μ deep where well developed, composed of clearly palisaded, elongate-clavate basidial husks, as much as 30 μ long, and nodose-septate hyphae 2–3 μ in diameter, with walls firm to more or less pronouncedly thickened, questionably amyloid, only thin outlining membranes stained by Congo red, rest of wall apparently more or less soluble in 10% KOH. CYSTIDIA coarse, as much as 120 μ long and emergent half-way, subcylindric, (5.5)7–8.5(10) μ wide just above the basal spread, obscurely furcate but sometimes showing a true bi-rooting, often broadening somewhat midway with a surface coating of crystalline mass, then narrowing slightly upward to a neck diameter of (5)5.5–8.8 μ, and extending to a more or less cylindric or gradually tapering but obtuse apex, (7.5)12–20 × (4.3)5–7 μ; wall smooth except where coated with crystalline mass, heavily thickened below with lumen capillary to occluded, beginning to thin just below the neck with a concomitant more or less abrupt expansion of the lumen into the thin-walled apex; amyloid particularly in circumluminar layer which fans out upward; middle layer of wall sensitive to and dissolving in KOH, thin outer and inner membranes apparently insoluble and staining lightly in Congo red. BASIDIA subcylindric to clavate, 16–30 × 4–4.5 μ, tetrasterigmate; basal walls more or less thickened. BASIDIOSPORES plentiful, slender cylindric, straight to curved, with curvature most pronounced at apicular end; (6.5)7.3–8.5 × (1.5) 1.8–2.2 μ; wall thin, smooth, non-amyloid.

To those of us who agree with Rogers and Jackson's (1943) decision that *P. glebulosa* (q.v.) is a *nomen confusum*, Donk's (1956*b*) designation of a taxon by that name as type species of his new genus *Tubulicrinis* is taken to mean that *P. gracillima* is that type. However, Donk has also given, as "type specimen", a Galzin collection No. *2261* (in herb. M.A.D. *2419*). If this specimen proves to be a collection of *P. gracillima*, there will be no problem. But should it prove to be something else—which may be the case, for the limits of this species are not yet certain—there will be further controversy.

[3]In these headings, names accepted, or concerning whose status no judgment is expressed, are printed in roman letters; synonyms, or names rejected for other reasons, are printed in italic type.

This is a nomenclatural issue which must be discussed separately lest it be buried in the taxonomic problem under present consideration: is a genus to be taken as typified by the species named as type species of that genus by the author, or is it necessary to examine the material studied by the author of the genus in order to discover whether or not he knew the species he named? One side of the issue has been argued by Donk (1952); and an attempt to deal with part of the question was made by Dostal *et al.* (1958) with their proposed amendment to Article 7 of the International Code, but the significance of the proposal apparently went unnoticed. It is hoped that some attention will be given, before the next Congress, to the differences in interpretation of Articles 7 and 9 as they affect generic typification, and that some clarification of the code will be introduced then.

Peniophora cretacea Bourdot & Galzin, Hym. de Fr. p. 288 ([1928])

This name is included herein only for the sake of comprehensiveness; it has been effectively interred as a ***nomen confusum*** by Rogers and Jackson (1943), and more recently by Donk (1956*b*).

Peniophora glebulosa (Fr.) Saccardo & Sydow, Syll. Fung. **16**, 195 (1902)
Thelephora calcea **glebulosa* Fr., Elench. **1**, 215 (1828)
Corticium glebulosum (Fr.) Bres., Fungi Trid. **2**, 61 (1898)

It is unfortunate that Dr. Donk (1956*b*), to whom I am indebted for friendly discussions on this point, has revived the name "*P. glebulosa*" after it seemed to have been successfully buried as a ***nomen confusum*** by Rogers and Jackson (1943). Donk's argument first challenges the validity of the publication of the Friesian "*glebulosa*" as a taxonomic unit, and then credits Bresadola with having supplied "a new name for a new taxon (or for a taxon in different rank)" when he published the new combination *C. glebulosum* (Fr.) Bres.

That Fries himself looked upon the asterisk with which he marked the italicized "*glebulosa*" as a sign of its taxonomic level is attested by his own hand on the labels of his own collections at UPS: "*Corticium calceum* v. *glebulosum*" (J. Nannfeldt, *in litt.*, to D. P. Rogers 1957). Bresadola (1898), in describing the taxon at the specific level, was at liberty to choose a new name and new type, but instead chose to raise the rank of the Friesian variety, using the Friesian name, and typifying it by Friesian material: "Specimina nostra cum speciminibus originalibus friesianis comparavi".

If all were well with this 'original specimen', there would probably have been no question raised regarding the validity of the publication of the Friesian name, nor would it have been considered pertinent that a raised rank "should not necessarily be considered a straight isonym" (Donk 1956*b*; but cf. Art. 49 and Recomm. 60-A-2 in Lanjouw *et al.* 1956). But when Bourdot and Galzin ([1913]) realized that there was something different between what they took for the Bresadola taxon and what "les mycologues suédois regardent comme le type friesien", they credited the first to Bresadola alone, as *P. glebulosa* Bres., and later ([1928]) separated out the second as *P. cretacea*, a clearly illegitimate action in view of Bresadola's explicit designation of the type of the name he used. And when it became known that the 'original specimen' (and therefore "*P. cretacea*") was actually (Litschauer 1938) a

combination of *P. romellii* and Bourdot and Galzin's interpretation of what was *P. glebulosa* in Bresadola's eyes, the only possible nomenclaturally legitimate course, "pour éviter toute nouvelle confusion" (Malençon 1954, p. 139), was taken by Rogers and Jackson (1943) when they discarded the *nomina confusa* based on the original specimen of Fries, and chose an indisputably new name, *P. gracillima* (cf. above), and a new type, for the controversial component.

The decision satisfied such workers as Boidin (1951*a*, 1951*b*, 1958*b*), Cunningham (1955), Malençon (1954), and Weresub (1953). But Donk's action (1956*b*) in designating, as the type species of his new genus *Tubulicrinis*, "*P. glebulosa* (Bres.) Sacc. & Syd.", followed by Eriksson's (1958) and Christiansen's (1960) acceptance of the name, has necessitated this restatement of what are, to us, the good reasons for discarding the epithet "*glebulosa*" in this context, along with its type and its variety of authorities and combinations.

Peniophora glebulosa var. *quercina* Donk, Mededel. Ned. Mycol. Ver. **18–20**, 165 (1931)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN M.A.D.
(on fallen branch of *Quercus robur;* Der Bilt. (Ut.); Aug. 1929; M. A. Donk *1994*)

FRUCTIFICATION creamy, soft cushiony, continuous; from a smooth, loose to tight mesh where thin and young, to a dense depth with punctate to almost grandinioid surface at maturity; distinctly hispid with cystidia. CONTEXT as much as, and more than, 100 μ deep [Donk: to 300 μ][4] where well developed; composed of clearly palisaded elongate-clavate basidial husks, and nodose-septate hyphae [Donk: 2–3.5 μ d.] with firm to more or less pronouncedly thickened walls, apparently non-amyloid; some cystidiform hyphae, 4 μ in diameter, with capillary lumen expanding near tip (usually burst open) with diameter broadened to as much as 4.8 μ. CYSTIDIA coarse, few complete for measurement of length, but frequently at least as much as 100 μ long, usually probably more [Donk: 125–236(300) μ], emergent, with stem more or less cylindric; 8–11 μ wide just above the basal spread, obscurely furcate, narrowing gradually, sometimes abruptly, to a neck diameter of 4.8–7.5 μ and extending to a more or less cylindric, obtuse apex, 10–12 $\times$ 7 μ (few apices seen); wall smooth, heavily thickened below with lumen capillary, beginning to thin just below neck with a concomitant more or less abrupt expansion of the lumen into the thin-walled apex; lightly amyloid, with amyloidity concentrated around lumen; middle layer of wall sensitive to and dissolving in 10% KOH. BASIDIA elongate-clavate, 19–25 $\times$ 4–5 μ [Donk: 22–30 $\times$ 4–4.5 μ], with four sterigmata erect, blunt and stout until maturity; basal wall more or less thickened. BASIDIOSPORES plentiful, frequently clumped, many collapsed; slender cylindric, straight to curved; 6.5–7 $\times$ 1.5–2 μ [Donk: 6–9 $\times$ 2 μ]; wall smooth, thin, non-amyloid.

This variety is of particular interest because it conforms so closely to the characters of the type specimen of *P. gracillima* (q.v.) while, according to a note enclosed in the envelope, apparently in Bourdot's hand, it differs from Bourdot's concept of "*P. glebulosa*" "par la teinte plus blanche, et ne paraît pas glébuleux". I feel it can safely be placed in synonymy with *P. gracillima*.

PENIOPHORA ANGUSTA Rog. & Weres., *in* Weresub, Can. J. Botany, **31**, 764 (1953)

DESCRIPTION OF TYPE COLLECTION IN TRTC
(on *Picea mariana;* Algonquin Park, Ont.; Aug. 25, 1939; R. F. Cain; TRTC *14469*)

FRUCTIFICATION white, effused, delicate, porose reticulate, distinctly hispid with cystidia; adherent to the substratum; margin indeterminate. CONTEXT sparse; subicular hyphae associated with substrate long-celled, branching at right angles to produce a trama of loosely aggre-

[4]Measurements so noted are taken from the packet or the published description of the designated specimen.

gated hyphal panicles, progressively shorter-celled; hyphae nodose-septate, 2.5 μ in width, with walls firm to sometimes more or less pronouncedly thickened, lightly stained by Congo red. CYSTIDIA slender, subcylindrical, (20)50–85 × (4)5–6 μ wide toward the furcate base, occasionally demonstrating true bi-rooting with distinctness, narrowing upward to a neck diameter of 4–4.5 μ, and extending to a narrowly cylindrical, obtuse apex 10–15(25) × 2.5–4 μ; wall smooth, faintly amyloid, heavily thickened below, beginning to thin just below the neck with a concomitant more or less gradual expansion of the basally capillary lumen, to culminate in the thin-walled apex one-eighth to one-half the length of the cystidium; middle layer of wall sensitive to and dissolving in 10% KOH, outer and inner membrane insoluble and lightly staining in Congo red. BASIDIA subclavate, 9–17 × 3.5–4.5 μ, bearing four slender, erect sterigmata, up to as much as 4 μ long. BASIDIOSPORES slender, cylindric; curved, straight, or sometimes slightly flexuous; 8–10.5 × 1.7–2–2.2 μ; wall thin, smooth, non-amyloid.

PENIOPHORA MEDIA Bourdot & Galzin, Bull. soc. mycol. France, **28**, 385 ([1913])

P. subalutacea ssp. *media* (Bourd. & Galz.) Bourd. & Galz., Hym. de Fr. p. 294 ([1928])

The original 1913 description was based on a specimen cited as "Printemps; sur pin; Causse Méjean (Lozère)". Such a collection is Bourd. *8924*, labelled "Champs. de la Lozère, No. *11468* des récoltes de M. Galzin, Envoi du 30 Mai 1912; sur pin; Truel (Causse Méjean)"—in PC. This is undoubtedly the specimen referred to, since the very brief description fits the material down to the point of failing to mention spores, which are certainly lacking in this collection.

DESCRIPTION OF PORTION OF TYPE COLLECTION IN PC
(Bourd. *8924*, Galz. *11468*)

FRUCTIFICATION milky cream, a closely knit but porose reticulum; separable. TRAMA an amyloid palisade of nodose-septate hyphae and basidial husks; wall emphasized or thickened, thickening on individual hypha or basidial base amyloid or not, more or less soluble in 10% KOH. CYSTIDIA subclavate to obtusely subconical, as much as 70 μ long, 5–6.5 μ wide just above the basal spread into what appears to be a true bi-rooting, narrowing more or less uniformly upward to a neck diameter of 4–5 μ, and extending to a more or less cylindric or expanded, obtuse apex, 10–18 × 4.5–6 μ; wall smooth, heavily thickened below with lumen capillary to occluded, beginning to thin usually well below the neck with corresponding gradual expansion of the lumen into the thin-walled apex; middle layer of wall sensitive to and dissolving in KOH, amyloidity concentrated in, but not exclusive to, the circumluminar layer; apices frequently collapsed, sometimes encrusted with resinous mass of crystals. BASIDIA tetrasterigmate, clavate, ca. 10×3.5 μ, seen mainly as more or less thick-walled basal husks, varying in amyloidity. BASIDIOSPORES not seen.

It is unfortunate that the holotype is incomplete, but as long as it is available with recognizable characteristics, it seems unnecessary to select a neotype. Certainly its cystidial and hyphal characters are clear enough to prove that the taxon has no affinity to *P. cineracea* or *P. subalutacea*, in spite of both Bourdot and Galzin's 1913 comment that it "relie *subulata* à *cineracea*", and its position in the 1928 publication as a "subspecies" under *P. subalutacea*. It is unquestionably a member of the "*P. gracillima* group", with cystidial characters apparently sufficiently distinctive for recognition of the taxon as a separate species.

The problem of spore character is, happily, not too difficult to solve, because a later collection (Bourd. *35988*, "sur pin, Bagnoler de l'Orne; VIII. 1923; E. Gilbert *687*"—PC), almost certainly among those cited in the 1928 publication, agrees in every detail with the type specimen and supplies, in addition, a few spores of the long, very slender, slightly curved type described in 1928 —as much as 8 μ in length, 1.5 μ in diameter.

Some explanation for the misplacing of *P. media* among the members of the *P. subalutacea* group may lie in a collection kindly lent to me by Leiden. Under their number L *930.58-26*, in Herb. L. Maire *127*, is a specimen labelled "Sept. 19, Andelot, affinis *glebulosa*. Recolte *1235* [or *1237*?]. C'est non le type commun. Notons *Pen. intermedia* p.t. Intermedium comme *Pen. media* B. & [G] est entre *glebulosa* et *subalutacea*". This notation seems to be in Maire's hand, possibly a copy of Bourdot's own comment on the specimen. In Leiden it is filed as *P. media*. And yet it is almost certainly a syntypic collection of *Odontia alutacea* ssp. *intermedia* Bourd. & Galz. ([1928], pp. 423–4), which is undoubtedly a member of the *P. subalutacea* group.

PENIOPHORA SUBULATA (Bourd. & Galz.) Donk, Mededel. Ned. Mycol. Ver. **18–20**, 165 (1931)

P. glebulosa ssp. *subulata* Bourd. & Galz., Bull. soc. mycol. France, **28**, 385 ([1913])

P. cretacea ssp. *subulata* (Bourd. & Galz.) Bourd. & Galz., Hym. de Fr. p. 289 ([1928])

A specimen in NY, Bourd. *6451* ("ad ligna carbonisata Pini, Causse-Noir, Aveyron; Galzin *2780*; 22.II.1908"), was undoubtedly among those used by Bourdot in his original description of this taxon ("ad ligna et cortices Pini sat frequens; Allier, Aveyron").

This is the only syntypic material that has been available to me. The collection is characterized by the presence of (*a*) strongly conical, non-amyloid 'lyocystidia', in width (5.5)8.5–12 μ just above the furcate, truly bi-rooted base, uniformly and rather sharply narrowing to a neck diameter of (3.7) 4.2–7 μ, topped by a more or less pointed arrowhead apex, 10–20 μ long, thin-walled through the abrupt expansion of the capillary lumen at the neck; (*b*) basidia with basally thickened, KOH-soluble walls that remain as closely packed husks in the trama, themselves broadly clavate on generally narrowed stalks with four sterigmata typically blunt, erect, and fingerlike before maturity; and (*c*) basidiospores that are slender cylinders, 7.5×1.5 μ.

These characters are quite clear, but the condition of Bourd. *6451* is poor on the whole, and difficult to study. Therefore, in the hope that syntypic material in better condition may eventually turn up, I prefer not to designate this particular collection as lectotype, leaving the species temporarily untypified. I think there is little risk of confusion, for *P. subulata* seems to be clearly distinct from other species in the group.

PENIOPHORA PROPINQUA (Bourd. & Galz.) Laurila, Ann. Botan. Soc. Zool. Botan. Fennicae Vanamo, **10**(4), 8–9 (1939)

P. cretacea ssp. *propinqua* Bourd. & Galz., Hym. de Fr. p. 288 ([1928])

The citations given for this taxon are as follows: "sur pin silvestre, Brefeld (Aveyron); Suède, C. G. Lloyd *8210*, *9122*". The Brefeld specimen has been unavailable in this study. Lloyd *08-210* (Bygget, Sweden, 1908), in PC, turns out to be a collection of *P. calothrix*. But Lloyd *09-122* (Smithsonian Collection *44589*, Bygget, Sweden, 1909), in TRTC ex BPI, conforms sufficiently to the description that it may be chosen as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE IN TRTC
(Lloyd *09-122*, Smithsonian *44589*)

FRUCTIFICATION creamy, densely reticulate, smooth to minutely mounded, continuous to cracked, distinctly hirsute in profile. TRAMA at least 50 μ deep, lightly amyloid, composed of nodose-septate hyphae up to 3 μ in diameter, some with thickened walls in part soluble in 10% KOH, along with basidial husks and cystidia. CYSTIDIA completely embedded or emergent, sometimes almost full length; subcylindrical, as much as 75 μ long, 5.5–8.5 μ wide just above the basal spread into a true bi-rooting or three roots, one false, narrowing slightly to a width of 5–7.5 μ somewhat below neck, reverting at the rather bulbous apex to a diameter of 5.5–8.5 μ; at maturity, wall smooth, heavily thickened throughout base and stem with capillary lumen abruptly dilating at the neck, rounding off its thin wall as economically as possible in a somewhat flattened bulb; middle layer of wall sensitive to and dissolving in KOH, amyloidity strong to weak, either throughout the wall or intensified in the circumluminar layer or in zones fanning out from near the base; apices often collapsed, collars and caps of resinous crystalline mass frequent. BASIDIA subclavate, with basal wall more or less thickened, thickening in part soluble, lightly amyloid; ca. 10 × 3.5–4 μ; tetrasterigmate. BASIDIOSPORES slender cylindrical, sometimes slightly broader at apex than at apiculate end, 7–8 × 1.2–1.5 μ; wall thin, smooth, non-amyloid.

PENIOPHORA CALOTHRIX (Pat.) Rogers & Jackson, Farlowia, **1**, 316 (1943)
Corticium calothrix Patouillard, Cat. Rais. Pl. Cel. Tunisie, p. 59 (1897)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN FH
(sur pin d'Alep, Bou-Chebka, Tunisie; Janvier 1893; N. Patouillard; *349*)

FRUCTIFICATION creamy, a densely knit reticulum, cushiony, smooth or somewhat punctate, continuous to glebulous; distinctly and regularly hispid; inseparable from crumbling substrate. Condition poor, difficult to study satisfactorily. SUBICULUM composed of few hyphae closely associated with the substrate, clamped, branching rarely at right angles upward, long-celled (to 60 μ long), 4–5 μ wide, with walls emphasized to thickened (to 1.2 μ wide). TRAMA composed of mainly emptied and collapsed, relatively short-celled, frequently branched hyphae, basidiophores, and husks of basidia, showing little if any sign of amyloidity, along with cystidia. CYSTIDIA subcylindrical throughout stem to the blunt or tapering, usually "hooded" apex; 50–60(85) μ long, 6–7.5(8.5) μ wide just above the bifurcate, typically truly bi-rooted base, narrowing only slightly and gradually to a neck width of 4.5–6 μ, then broadening slightly in the apex, 13–20 × 5.5–7.5 μ; wall variably amyloid, negatively to faintly to (usually) positively, smooth, thick, not colored by Congo red except perhaps in inner and outer membranes, middle layer swelling and dissolving in 10% KOH; lumen capillary in base and stem, with asymmetric expansion abrupt (rarely gradual) at the neck to form the (usually) one-sidedly thin-walled apex, the thickened wall generally extending like a hood along one side and over the summit; sometimes capped with topping of radiating chiplike crystals, or with amorphous mass surrounding apex. BASIDIA not seen; only squat husks of their basally thickened walls observable, not noticeably amyloid. BASIDIOSPORES not seen, but observed by D.P.R. who records them, in his personal notes, as obtuse, curved, cylindric, 7.5×2.5 μ.

Comparison of the above with the descriptions of type material of *P. delectans* and *P. pirina* (cf. below) throws doubt on their published synonymy with *P. calothrix* (Rogers and Jackson 1943; Weresub 1953).

PENIOPHORA DELECTANS Overholts, Mycologia, **26**, 513 (1934)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(on wood of dead conifer; Cook Forest, Clarion Co., Penn.; June 23, 1932; Overholts *16260*)

FRUCTIFICATION grayish white to creamy, a reticulum varying from fine to rather coarse in mesh, thin but building up to a measurable depth in part; distinctly and regularly hispid. SUBICULUM composed of few hyphae, long-celled, clamped, branching rarely at right angles, 3.5–4 μ wide, with walls emphasized to thickened (to 1.5 μ wide), showing no sign of amyloidity; tissue above, a disordered mass of mainly emptied, relatively short-celled, frequently branched hyphae, basidiophores, husks of basidia, and cystidia. CYSTIDIA with stems subcylindrical, 45–65 μ long, 6–7.5 μ wide just above the bifurcate, typically truly bi-rooted base, narrowing almost imperceptibly to a neck width of 4.8–6 μ, then extending into a cylindrical or somewhat tapered apex, 12–15 × (3.5)4.8–6 μ; wall amyloid, smooth, thick, middle layer swelling and dissolving in 10% KOH, not colored by Congo red except perhaps in inner and outer membranes; lumen capillary in base and stem, with asymmetric expansion abrupt

(rarely gradual) at the neck to form the (usually) one-sidedly thin-walled apex, the thickened wall generally extending like a hood along one side and over the more or less tapered summit; more or less encrusted at the apex, sometimes capped with crystals. BASIDIA short and squat, 8–11 × 3.8–4.5 μ, tetrasterigmate, with basally thickened walls soluble in KOH, their husks occasionally showing some suggestion of amyloidity. BASIDIOSPORES rare, 5.4–6.5 × 1.8 μ; cylindrical, slightly curved; wall thin, smooth, non-amyloid; not staining strongly in phloxine, perhaps containing little cytoplasm, possibly subnormal in size.

Although previously (cf. Rogers & Jackson 1943; Weresub 1953) placed in synonymy with *P. calothrix*, this taxon may perhaps be revealed as different, if collections can be divided into two groups—one with fructifications paler, thinner, and with narrower spores (*P. delectans*), the other with creamier, deeper, denser fructifications and somewhat broader spores (*P. calothrix*). This remains to be checked. Meanwhile I can only say that the types of the two taxa are not, to me, immediately identifiable as one species, although the differences may be inconsequential, perhaps due in large part to the absence of a sufficient number of normal spores in the two collections.

PENIOPHORA PIRINA Bourdot & Galzin, Bull. soc. mycol. France, **28**, 387 ([1913])

The host and locale of the original description are: "sur poirier, Aveyron". The 1928 description adds "été". Comparison of the descriptions in the two publications leads one to believe that only one specimen was at hand for both. This appears to be a collection in PC, labelled in Bourdot's hand: "Bourd. *6404*; Champs. de l'Aveyron; No. *4092* des récoltes de M. Galzin; VI. 1909; sur Poirier, debout Vignoles; *P. pirina*, Hym. de Fr. no. 266".

DESCRIPTION OF PORTION OF TYPE COLLECTION IN PC
(Bourd. *6404*, Galz. *4092*)

FRUCTIFICATION from thin white sprinkle to yellowing, densely knit, cushiony depth, continuous to glebulous, more or less separable. SUBICULUM composed of clamped, long-celled, sparingly branched hyphae up to 4 μ in diameter, with walls thickened to as much as 1 μ in width, sometimes agglutinated into ropes basal to the fructification; tissue above compact, composed of distinct hyphae with firm to thickened walls, branching profusely in candelabrum arrangement for the production of distinct basidiophores and basidia in an evenly palisaded hymenium; all thickened walls clearly amyloid. CYSTIDIA subcylindrical or subconical in the stem, 55–85 μ long, 5–6.5(7.5) μ wide just above the forking base (frequently truly bi-rooted, sometimes apparently with three or four 'true' roots), narrowing upward gradually to a neck width of 4–5.5 μ, then cylindrical or slightly expanded in the apex; wall distinctly amyloid, especially in the circumluminar layer, smooth, thick, middle layer swelling and dissolving in 10% KOH, not colored by Congo red, while inner layer and outer membrane resistant to KOH and stained by Congo red; lumen capillary in base and part of stem, expanding gradually to form a relatively thin-walled, rounded or blunt apex, obpyriform to oblong elliptical, 8–25 × 4.5–8 μ rarely with a suggestion of one-sided thickening of the wall but not hooded, occasionally topped by cap of crystals. BASIDIA clavate to subcylindrical, more or less elongate, with truncate apex, tetrasterigmate, 12–25 × 4–5 μ, with basal wall usually thickened, generally amyloid. BASIDIOSPORES broadly cylindrical, barely curved, 5–6–7 × 2–2.7 μ; wall thin, smooth, non-amyloid.

When Rogers and Jackson (1943) transferred Patouillard's *Corticium calothrix* to *Peniophora*, they placed the above name in synonymy with it; but since they do not mention having seen either type or authentic specimens of *P. pirina*, it is likely that they formed their concept of this species through European collections misdetermined as *P. pirina* by mycologists other than Bourdot. By comparing this description with that of the type of *P. calothrix*, we can see what may be noteworthy differences in the structure of the fructi-

fication, the cystidia, and basidia, as well as the choice of host. It now seems doubtful that the identity of *P. pirina* can be submerged in that of *P. calothrix*.

PENIOPHORA EFFUGIENS Bourdot & Galzin, Bull. soc. mycol. France, **28**, 386 ([1913])

The original short description is clearly applicable to a PC specimen labelled by Bourdot: "Bourd. *8719*; Champs. de l'Aveyron; *11060* des recoltes de M. Galzin; 19.III.1912; sur châtaignier, Forques". A notation on the packet regarding measurements of cystidia ("74–95×6 μ") and of spores ("6×3–3.5 μ") coincides exactly with those in the 1913 description. This is unquestionably the type collection.

DESCRIPTION OF PART OF TYPE COLLECTION IN PC
(Bourd. *8719*, Galz. *11060*)

FRUCTIFICATION creamy, coarsely porose reticulate, appearing tufted; distinctly hispid; more or less separable. Each tuft composed of compactly intertwined hyphae, leading to a more or less conglutinated hymenium, difficult to separate into components. HYPHAE clearly nodose-septate, generally 3 μ in diameter, with walls firm to considerably thickened, eventually staining in Congo red, but not throughout; trama, where deep, largely composed of basidial and hyphal husks. CYSTIDIA numerous, variable, (40)50–80 to over 110 μ in length, conical, or when more or less cylindric in the stem then with sharply tapered apex; 3–6 μ in diameter at the basal septum or just above the basal spread, 4–4.5 μ in stem diameter, narrowing somewhat to an indefinite neck 3.3–4.5 μ wide, about 20–30 μ from the tip, apex pointed or with rounded taper or almost cylindrically 3 μ in diameter; originating frequently as direct apical extension of cystidiophore, sometimes bifurcate, with no evidence seen of true bi-rooting; wall non-amyloid, thickened at least in lower half of stem, the middle layer soluble in 10% KOH, unstained by Congo red, the outer membrane and inner layer insoluble and colored by Congo red; lumen generally capillary below, expanding gradually from about half way to the thin-walled apex. BASIDIA more or less clavate, 15–22 × 5 μ (from few measurements), basal wall uniformly but narrowly thickened, remaining as husk after maturity; tetrasterigmate. BASIDIOSPORES variable, ellipsoid to oblong, adaxially flattened to incurved, occasionally with an askew taper to the apiculus; 5–6.5 × 2.5–3.3 μ; wall thin, smooth, non-amyloid.

PENIOPHORA SUBALUTACEA ssp. ATTENUATA Bourdot & Galzin, Hym. de Fr. p. 294 ([1928])

This is an excellent example of the difficulties involved in assigning a subspecific status to the taxa in this group. "Slanted type" indicates its rank, and position below *P. subalutacea* leads one to understand that Bourdot and Galzin meant it to be subspecific to that species. The citation is "Septembre à Novembre. Sur bois caries de châtaignier, Aveyron". A request for "*P. attenuata*" brought from PC a collection whose packet is labelled in Bourdot's hand: "Bourd. *12154*; Champs. de l'Aveyron; *13844* des récoltes de M. Galzin; Envoi du 16.IX.1913, sur châtaignier, Loubotis; *P. hirtella* v. *attenuata* B.". The sketch which accompanies this collection is not identical with that published, but is very close; and the observations noted on the packet tally with the published description: on the packet—"sp. 7–8 × 2.5–3 μ. Cyst. a p. mince supérieurement, 90-120 × 6 μ très aigue"; and in publication—"Sp. 7–9 × 2–3 μ; Cyst .... 90–120 × 6 μ, à parois épaisses dans les deux tiers inférieures et insensiblement amincies jusqu'au sommet très aigu". The collection is certainly representative of this taxon.

Bourdot and Galzin's footnote to their description explains the reason for the difference in name between packet and publication: "Les cystides sont quelquefois moins longuement attenuées et la plante se rapproche de *P. hirtella*". The taxon was probably originally thought to be closer to *P. hirtella*.

And certainly this collection is confirmation of that relationship rather than of any affinity to *P. subalutacea*.

There is one jarring note: the hyphae are reported "à cloisons fréquentes, sans boucles", whereas the hyphae in this specimen are certainly nodose-septate. However, this discrepancy cannot be taken to negate the right of this collection to be chosen as lectotype. It is clearly a mistake—by a man who, although using magnifications far lower than most of us, made so few errors in observation that one forgets it is possible for him, too, to be wrong. It may also be that this description is based on a confusion with *P. hirtella* in which the clamps are less conspicuous than in this taxon.

Taking all these factors into consideration, I feel it justifiable to designate this specimen as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN PC
(Bourd. *12154*, Galz. *13844*)

FRUCTIFICATION white to creamy, effused, coarsely porose reticulate, appearing tufted; distinctly hispid; more or less adherent. Apparently composed of more or less closely compacted individual tufts, each a rather tightly branching unit arising from a single upright hypha, a branch of the sparse horizontal subicular hyphae. HYPHAE clearly nodose-septate, 2.3–3 μ in width, with walls firm, eventually staining in Congo red; occasional cystidial hyphae present, ca. 3 μ wide, thick-walled to the point of capillarity of the lumen, often extending a length of as much as 250 μ without a noticeable septum. CYSTIDIA numerous, (45)65–85 to over 120 μ in length, typically more or less subcylindric in the lower part of the slender stem, 3–4–5.5 μ in width, usually tapering somewhat to an indefinite neck 3–4.5 μ in width, then more sharply attenuate; or conical, tapering uniformly from base to pointed tip, or with apex, (12)15–25 μ long, less frequently slightly expanded, somewhat ovoid, or cylindric and obtuse; originating sometimes as direct extension of central hypha of clump, but more frequently bifurcate, usually falsely bi-rooted, occasionally truly so; wall non-amyloid, thickened at least in the lower half of the stem, the outer membrane and inner layer insoluble and colored by Congo red; lumen generally more or less capillary below, expanding gradually to the thin-walled apex. BASIDIA flexuous cylindrical, 15–20 × 5–6.5 μ (from few measurements), basal walls uniformly but narrowly thickened, some remaining as husks after maturity; with four erect, slender sterigmata up to 7 μ long. BASIDIOSPORES ovoid-ellipsoid to broadly cylindrical, adaxially somewhat flattened to incurved, frequently with an askew taper to the prominent apiculus; generally not fully stained by phloxine, suggesting a reduced cytoplasmic content and perhaps an incipient shrinking that could account for the variations in shape; 6–7(8) × 2.5–3 μ; wall thin, smooth, non-amyloid.

As may be seen by comparing this description with that of the type of *P. effugiens*, these taxa are very close. The differences are slight: an occasional true bi-rooting and a slightly narrower spore here. Almost certainly the two may be considered one species. But I shall withhold the decision until a report is made on additional collections which can help assure the synonymy.

PENIOPHORA PROMINENS Jackson & Dearden, Mycologia, **43**, 57 (1951)

Although the original description is based on a single specimen and therefore delineates the type collection alone (on rotting wood of *Pinus monticola;* Bonner Co., Idaho; June 12, 1940; A. W. Slipp, ID For. Path. Herb. *2418*), it seems advisable here to emphasize certain characteristics which may eventually be useful in determining the relationships of this taxon.

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(ID For. Path. Herb. *2418*)

FRUCTIFICATION white to cream, delicate, discontinuous, formed of loosely aggregated floccose tufts, delicately hispid, easily separable. No continuity to subiculum, composed of few and isolated basal hyphae, horizontal, nodose-septate, with walls more or less thickened, part of thickening soluble in KOH, part insoluble and stainable in ammoniacal Congo red; these hyphae giving rise at intervals to upright branches terminated by basidia clustered

around a single cystidium or grouped cystidia. CYSTIDIA cylindrical or slightly tapering or expanding above, 60–110 μ long, 3–4.8 μ in diameter at basal septum; arising as direct extensions of cystidiophoric hyphae; wall non-amyloid, thickened below with capillary lumen expanding gradually to a relatively thin-walled upper third, 3–5.8 μ in diameter; inner layer of wall relatively broad (especially toward tip, where as much as 1.2 μ wide), outer layer thin, both colored strongly by Congo red, middle layer unstained and soluble in strong alkali. BASIDIA like broad-stemmed bulbs, 15–20 × 4.5–5.5 μ, bearing four subulate, slightly arcuate sterigmata, these frequently collapsed in mount with spores apparently sitting directly on basidial apex; wall thickened below: "after maturity clusters of basidial bases remain as stalked cyathiform structures with thick rigid walls, proliferation of basidia . . . acropetal through subtending clamps" (Jackson and Dearden *loc. cit.*). BASIDIOSPORES subglobose, 3.5–4 × 3.5–4.8 μ, with a conspicuous lateral peglike apiculus; wall thin, smooth, non-amyloid.

The three taxa just considered approach each other in several ways, particularly in structure of fructification and origin of cystidia. Whether these similarities, by which they differ from other members of the group, are significant enough to require their exclusion from the genus *Tubulicrinis* remains to be decided.

PENIOPHORA HIRTELLA Bourdot & Galzin, Bull. soc. mycol. France, **28**, 386 ([1913])

An authentic specimen (in S) is labelled in Bourdot's hand: "Bourd. *8717*; Champs. de l'Aveyron; *11008* des récoltes de M. Galzin; Envoi du 19.III.1912; sur châtaignier; les Vives; *P. hirtella* B. & G. IV, n. 263". This collection is undoubtedly syntypic, for the description fits it closely, except for the words used to characterize the spores—"oblongues-claviformes, un peu arquées"—which hardly describe a spore that is typically slightly narrowed to the apical as well as the apiculate end. However, there is no question that this specimen truly represents the species, since the published sketch illustrates the actual appearance of the spores. Bourd. *8717* is therefore selected as lectotype for this species.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN S
(Bourd. *8717*, Galz. *11008*)

FRUCTIFICATION creamy, effused, a delicate reticulum, distinctly hispid with emergent cystidia; inseparable from substrate. CONTEXT shallow, composed of distinct nodose-septate hyphae, 3.5–5 μ in diameter, with wall pronounced. CYSTIDIA emergent, frequently for most of length, as much as 75 μ tall, with stems almost precisely cylindrical, measuring 4–6.5 μ just above the furcate base and ca. 3.5–5 μ at the neck; lumen capillary in stem, expanding abruptly at the neck to form an elongate, thin-walled apex, 13–25 μ long, typically cylindrical for more than half that length, 3.5–6 μ broad, then usually tapering abruptly to a mucronate tip; wall non-amyloid, thickened throughout stem, the middle layer soluble in 10% KOH, unstained by Congo red, the outer membrane colored by Congo red; true bi-rooting with attached cystidiophores frequently distinct; apex often covered by agglutinated mass of spores. BASIDIA clavate, 10–18 × 4.3–5.8 μ, with basal wall narrowly thickened, and four slender sterigmata. BASIDIOSPORES more or less flexuous subfusoid to subellipsoid, slightly curved, 6.2–7–9 × (1.8)2–2.8 μ; wall thin, smooth, non-amyloid.

PENIOPHORA INORNATA Jacks. & Rog., *in* Jackson, Can. J. Research, C, **26**, 139 (1948)

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN TRTC, DAOM
(on pine; Timagami, Ontario; Aug. 11, 1939; H. S. Jackson, TRTC *15004*)

FRUCTIFICATION varying from a sparse creamy hispidity to a delicate network; inseparable. CONTEXT composed of distinct hyphae, firm-walled, nodose-septate, up to 3 μ in diameter; branching frequently right-angled; subiculum of few horizontal hyphae from which arise cystidia or erect branches producing cystidia and basidia, with little build-up of trama. CYSTIDIA up to 70 μ long, 4–5.5 μ wide, at maturity characteristically looking like rifles with bayonet-insertions, the stem more or less cylindrical, thick-walled with more or less capillary lumen, this extending above the neck as a slender, thin-walled projection as much as one-third to

one-half the full length, naked or typically capped or one-sidedly shielded by an acutely pointed conical "dunce-cap" of different composition, not as readily soluble in alkali as the thickened walls of the cystidial stems; cystidial base usually distinctly bi- or even tri-furcate, arising from a horizontally placed hypha, frequently but not always truly bi-rooted, sometimes clearly the apical extension of an erect or simply horizontally deflected hypha; thickened wall non-amyloid, the middle layer swelling drastically and eventually dissolving in 10% KOH and unstained by Congo red, inner and outer membranes insoluble and lightly colored by Congo red, but outer usually burst by pressure from the swelling middle layer. BASIDIA flexuously cylindrical, 10–15 × 4–5 μ, with four slender, erect sterigmata ca. 3.5 μ long. BASIDIOSPORES subgloboid to oblong or ovate, 4.2–5.5 × 3–3.5 μ; wall thin, smooth, non-amyloid.

Although *P. inornata* could be mistaken for a young fructification of *P. chaetophora* in gross appearance, its distinctive cystidia allow for no confusion of the two once microscopic examination is made.

PENIOPHORA CHAETOPHORA (Hoehn.) Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **115**(I), 1606 (1906)

Hypochnus chaetophorus Hoehn., *ibid.* **111**(I), 1007 (1902)

Undoubtedly syntypic and quite likely to be the only specimen von Hoehnel had on hand when he described this taxon is the collection lent me by Farlow and labelled "auf morsch. N.H., Krummbachleithen, Schneeberggebiet; 24/7, 1902; v. Hoehnel". It is here designated as lectotype, though it may actually be holotype.

DESCRIPTION OF PORTION OF LECTOTYPE IN FH
(No. ? Hoehn. *636*)

FRUCTIFICATION white to cream; coarsely reticulate, prominent cystidia giving it a sponge-spiculate appearance from youth to age; separable in tufts around individual or groups of cystidia. SUBICULUM very sparse, composed of few substrate-associated hyphae, firm-walled, distinctly clamped, 2.5–3 μ in diameter; trama almost non-existent in young portions of fructification, where tissue is massed only around and up the cystidia, but building up in older portions between early-formed cystidia; tramal hyphae apparently indistinguishable from subicular except for presence of some cystidiform hyphae, thick-walled with capillary lumen, ca. 3.5 μ in diameter; walls of ordinary hyphae stained by Congo red. CYSTIDIA typically conical, with sprinkling of occasional cylindrical or subclavate types, these usually immature; at maturity mainly 100–200 μ long, 7.5–12 μ in diameter above the usually truly bi- to multi-rooted base; typically tapering uniformly to a pointed apex; some hyphal cystidia also present, tapering upward from a base ca. 3.5 μ in diameter; wall smooth, thick, generally more or less strongly amyloid, but varying from not at all amyloid through complete range; amyloidity concentrated in the circumluminar or median layer; Congo red staining rare, and then only in thin outer membrane; rest of wall soluble in 10% KOH; lumen typically capillary throughout, but occasionally broader below, or expanding gradually in stem, or abruptly almost at tip; frequently open at tip when mounted, perhaps because abrupt swelling of cytoplasm bursts thin tip-wall. CYSTIDIOLES (or basidioles?) more or less capitate, 15–25 × 3–5 μ at base, with head 3.5–4.2 × 4.5–5.5 μ, sometimes haloed by an oily mass; basal wall of stem slightly thickened, occasionally with suggestion of amyloidity; wall staining lightly in Congo red; frequently rising perpendicular to originating hypha, but with basal clamp. BASIDIA cylindrical to subclavate, more or less flexuous, somewhat corseted[5]; 15–25 × 4–5.5 μ; wall staining lightly in Congo red; with four erect, arcuate sterigmata, ca. 5 μ long. BASIDIOSPORES ellipsoid-oblong, with granular cytoplasm; 5.5–6 × 2.8–3.3 μ; wall thin, smooth, non-amyloid.

Peniophora abnormis Bourdot & Galzin, Hym. de Fr. p. 290 ([1928])

The description of this species is apparently based on a single specimen: "Septembre; sur bois de sapin pourri; Turini, Alpes Mar. (E. Gilbert)" (Bourdot and Galzin *loc. cit.*), which is probably the PC collection labelled: "Bourd. 39513♀; Champs. des Alpes Mar.; *Peniophora abnormis* n. sp.; sapin, Turini; legit. Gilbert *1220*; IX, 1925". This number is therefore type.

As has been said before (Weresub 1953), there is nothing in the description

[5]Corseted is a term for which I am indebted to A. M. (Mrs. D. P.) Rogers.

to distinguish this taxon from *P. chaetophora* except (*a*) the different terms used to describe the spores—in *P. chaetophora* "largement ellipsoïdes-subcylindriques" and in *P. abnormis* "ovoïdes ou elliptiques, légèrement déprimées d'un côté"—and (*b*) the postscript notation: "Hyphes et cystides absorbant l'éosine, ce qui est très rare dans les espèces ayant des cystides à canalicule capillaire".

Examination of the specimen cited yields no information that would lead to its segregation from *P. chaetophora*. The fructification is coarsely hispid, separable, and spongy. The cystidia generally stretch through the full depth of the fructification and well above it, up to 200 μ long and 10 μ wide, with capillary lumen usually throughout their conical length, in some cases with expansion of the lumen at the tip, as illustrated by v. Hoehnel and Litschauer for *P. chaetophora* (1907, p. 749). The walls of most cystidia are non-amyloid, but in a few, a layer close to the lumen is strongly amyloid, and in others almost the entire thickness reacts positively to Melzer's. The thin outermost layer of the wall stains in Congo red (to which, apparently, tissues react as they do to eosin), but such is the case in *P. chaetophora* as well. The spores average 5×3 μ, which is the norm for *P. chaetophora*, and their shape is equally characteristic of that species.

It appears therefore that *P. abnormis* is synonymous with *P. chaetophora*.

Peniophora dissoluta Overholts, Mycologia, **26**, 514 (1934)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(on wood of conifer; Center Co., Penn.; Apr. 23, 1933; W. L. White & C. Bidwell; Overholts *16264*)

FRUCTIFICATION pure white, a broadly effused reticulum, coarse in mesh, sponge-spiculate with prominent cystidia; separable in tufts around individual or groups of cystidia. SUBICULUM inconspicuous, composed of substrate-associated hyphae, firm-walled, distinctly clamped, 2.5–3.5 μ in diameter; trama and hymenium well developed, but all structures strongly agglutinated and difficult to separate; some hyphae with a suggestion of questionable amyloidity in emphasized walls; cystidiform hyphae present, thick-walled with capillary lumen, 1.8–2.5 μ in diameter. CYSTIDIA typically conical, with a sprinkling of occasional cylindrical or subclavate types, these usually immature; at maturity as much as 180 μ long, 7.3–11 μ in diameter above the usually truly bi- to multi-rooted base; typically tapering more or less uniformly to pointed apex; some hyphal cystidia also present, tapering upward from a base 2.5–3.5 μ in diameter; walls smooth, thick, quickly soluble in 10% KOH, generally more or less strongly amyloid, but varying from not at all amyloid through complete range; lumen typically capillary throughout, but sometimes broad in part or throughout. Agglutinated hymenium difficult to separate into components, with suggestion of capitate cystidioles (?) but not clear. No mature BASIDIA seen; basidioles more or less cylindrical, ca. 20 × 5 μ. BASIDIOSPORES very rare, ellipsoid-oblong, 5.5×2.8 μ; wall thin, smooth, non-amyloid.

P. chaetophora is so highly characteristic in gross and microscopic morphology that there can be little doubt that *P. dissoluta* is, as was pointed out by Rogers and Jackson (1943), synonymous with it.

PENIOPHORA ACCEDENS (Bourd. & Galz.) Wakefield & Pearson, Trans. Brit. Mycol. Soc. **6**, 140 ([1919])

P. glebulosa ssp. *accedens* Bourd. & Galz., Bull. soc. mycol. France, **28**, 386 ([1913])

The original description places this fungus "ad ligna putrida Castaneae frequens, rarior ad Quercum". Four authentic collections of likely syntypic date have come to hand: Bourd. *6524* ("châtaignier très humide, Casourgues, Tarn, Galzin *4307*, 23.VII.1909"—S and UPS); Bourd. *8780* (=*8691*) ("sur

châtaignier, Loubotis, Aveyron, Galzin *11137*, IV.1912"—NY and TRTC); Bourd. *30589* ("châtaignier, Bois du Fabre, Aveyron, Galzin *11291*, 20.IV. 1912"—L *930.58-33*); and Bourd. *8701* ("sur châtaignier, Guèrgues, Aveyron, Galzin *11028*, 19.III.1912"—M.A.D. *2420*).

The latter collection proves to be different from the others, its cystidia broader and more distinctly conical in the stem, and its spores cylindrical—closer to *P. juniperina* or, perhaps better still, to *Tubulicrinis borealis* J. Erikss. Of the other three collections listed, the first, Bourd. *6524*, displays on its packet Bourdot's measurements which coincide almost to the point of identity with those given in the 1913 description. It is therefore chosen as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN S, UPS
(Bourd. *6524*, Galz. *4307*)

FRUCTIFICATION white, a very delicate but compact reticulum, closely adherent, margin indeterminate; surface pruinose, minutely setulose. CONTEXT sparse, composed of clamped, thin-walled hyphae, 2–3 μ in diameter, with erect branches rising perpendicular to repent hyphae. CYSTIDIA self-capitate, with slender, subcylindrical stems, 50–65 $\times$ (3)3.5–4.8 μ just above the furcate base, apparently only sometimes truly bi- to several-rooted; narrowing to a neck width of 2.5–3.3 μ; wall non-amyloid, smooth, thick, middle layer swelling and dissolving in 10% KOH, not colored by Congo red; thin outer membrane staining in Congo red; lumen capillary in base and stem, expanding abruptly at neck to form thin- to firm-walled, Congo-red-stained apical bulbs 6.5–8.5 $\times$ 6.5–8 μ, sometimes haloed by an amorphous mass. Occasional cystidia cylindrical to subconical, lacking bulb, lumen not capillary except in roots or not even there, relatively broad in stem, with wall thinning gradually to elongate, thin-walled apices. BASIDIA clavate, with clamp at base, generally produced in candelabrum clumps, though sometimes as direct erect branches from repent hyphae; 9–12 $\times$ 4–4.5 μ [Bourdot: 12–15 $\times$ 4–4.5 μ]. BASIDIOSPORES broadly ellipsoid with slightly flattened adaxial side, 4–5 $\times$ 2.7–3.2 μ [Bourdot: 4–4.5 $\times$ 3–3.5 μ]; prominently apiculate; wall thin, smooth, non-amyloid.

PENIOPHORA THERMOMETRA Cunningham, Trans. Roy. Soc. N.Z. **83**, 292 (1955)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(on *Metrosideros robusta;* Auckland, N.Z.; May 1952; G. H. Cunningham, P.D.D. herb. *11483*)

FRUCTIFICATION barely observable, delicate white mesh, to more clearly distinguishable yellowish network, minutely setulose with cystidia; completely inseparable from crumbling substrate. CONTEXT very sparse, apparently composed of few repent substrate-invading hyphae, 2–2.8 μ in diameter, nodose-septate, thin-walled, frequently collapsed, with few right-angled branches; practically no build-up of trama. CYSTIDIA self-capitate, with slender, subcylindrical stems, 40–65 $\times$ 3–5 μ just above the furcate base [Cunningham: 45–64 $\times$ 4–6 μ], frequently clearly truly bi-rooted, sometimes several-rooted; narrowing to a neck width of 2.5–3.8 μ; wall non-amyloid, smooth, thick, middle layer swelling and dissolving in 10% KOH, not colored by Congo red; thin outer membrane and firm inner layer staining in Congo red; lumen capillary in base and stem, expanding more or less abruptly at neck to form firm-walled, Congo-red-stained apical bulbs 5.2–7 $\times$ 4.8–7 μ; sometimes haloed by amorphous mass or loaded with conglutinated spores. Occasional cystidia subcylindrical throughout, lacking bulb, lumen capillary only half way in stem, if at all, with wall thinning gradually to elongate, thin-walled apices. Few clear BASIDIA seen, somewhat clavate, or subpyriform on short stalk, or flexuous cylindrical, 6.5–10 $\times$ 4–4.5 μ [Cunningham: 6–8 $\times$ 4–5 μ], bearing four sterigmata, arcuate at maturity; relationship to basidiophore obscure. BASIDIOSPORES abundant in masses on cystidia and on substrate; globose, 3.2–4.3 μ [Cunningham: 3.5–4 μ] in diameter, to subglobose, rarely obovoid, 4–5 $\times$ 3.8–4.5 μ; apiculus prominent; wall thin, smooth, non-amyloid.

My observations on this collection, after days of study, are less than satisfactory. Only the cystidia and the spores are in condition for detailed description. Though basidia from the basidiole to the sterigmate stage are present, none was found bearing mature spores, in spite of the masses of spores throughout; nor could I convince myself of the manner of origin of the basidia,

which sometimes give the impression of a pleurobasidiate (Donk 1956*a*) structure. To confuse the picture further, patches of the yellowing fructification bear tulasnellaceous basidia with exhausted epibasidia. However, Cunningham's (*loc. cit.*) observations definitely link the globoid spores with the homobasidia present. As pointed out by him, the globoid spores differentiate this taxon from *P. accedens*. Christiansen's (1960) report of *P. thermometra* from Denmark, however, gives dimensions that more nearly approach those of *P. accedens*.

PENIOPHORA SCEPTRIFERA Jacks. & Weres., *in* Weresub, Can. J. Botany, **31**, 772 (1953)

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN TRTC, DAOM (on leafy liverwort and rotten conifer log; Algonquin Park, Ont.; Sept. 1, 1939; R. F. Cain, TRTC *14794*)

FRUCTIFICATION a white, very delicate reticulum, minutely setulose, closely adherent to substrate. CONTEXT sparse, composed of clamped, thin- to firm-walled hyphae, 1.5–2.8 μ wide, with erect branches rising perpendicular to repent hyphae; some, acting as basidiophores, as much as 3.8 μ broad. CYSTIDIA self-capitate, very slender, 35–65 × (1.5)2.5–3(3.5) μ wide just above the furcate base, apparently only sometimes truly bi-rooted; narrowing gradually or tapering sharply (latter especially where fungus grows on the liverwort) to a neck width of 0.8–2.8 μ; wall non-amyloid, smooth, much or little thickened, middle layer swelling and dissolving in 10% KOH, not colored by Congo red; outer and inner membranes insoluble and Congo-red-stainable; lumen narrow or capillary in base and stem, broadening gradually or abruptly at the neck, usually flaring into prominent thin- to firm-walled, Congo-red-stained apical bulbs 4–7 × 4–6.5 μ, or bulbs lacking, sometimes replaced by thin-walled cylinders; sometimes haloed by amorphous mass or conglutinated spores. BASIDIA clavate or like broad-stemmed bulbs, typically with clear clamp at base whether arising directly from repent hyphae or terminating basidiophoric branches; generally solitary; 10–18 × 4–5.5 μ, with four slim, arched sterigmata as much as 4 μ long. BASIDIOSPORES broadly ellipsoid or subovoid, slightly incurved adaxially, 5–6.5 × 3–4.5 μ; prominently apiculate; wall thin, smooth, non-amyloid.

Here, as in the case of *P. thermometra*, there is great difficulty in assuring oneself of the kinship of hyphae, cystidia, basidia, and spores, when they occur together with extraneous hyphae etc. on the crumbling wood substrate. Fortunately, parts of the fructification are overgrowing a leafy liverwort which provides an ideal setting for clarity of structure. The original *Latin* description was based on the growths occurring only on the liverwort, but the picture here includes those parts of the fructification which occur on the wood of the type collection, since they are judged to be the same species, though demonstrating some variability in structure.

PENIOPHORA JUNIPERINA (Bourd. & Galz.) Bourdot & Galzin, Hym. de Fr. p. 289 ([1928])

P. glebulosa ssp. *juniperina* Bourd. & Galz. Bull. soc. mycol. France, **28**, 386 ([1913])

In their original subspecific description of this taxon, Bourdot and Galzin gave the following information regarding habit, habitat, and locale: "frequens ad ligna et cortices Juniperi, rarior ad Pinum, Ericam. Allier, Aveyron, Tarn. . . . Bien distinct par ses cystides et les papilles de l'hyménium".

Of the possible syntypes (pre-1913 collections) which have come to my attention, five are on juniper: Bourd. *31897* (in UPS), collected Nov. 29, 1912, was probably not at hand for the 1912 preparation of the description; Bourd. *8035* (in M.A.D. *2416*) is largely overgrown by a hyphomycete; Bourd. *8038* (in L *930.58-44*) appears to lack spores; Bourd. *8040* (in BPI) seems to lack

any indication of a papillate hymenium; and Bourd. *20860* (in FH), although collected by Bourdot himself in May 1911, is suspect in that its large accession number suggests that it was not studied until some time after the other 1911 collections, perhaps only after the 1913 publication was in press or even in print. All this makes the choice of a lectotype from among these specimens of questionable value, although all appear to be conspecific according to our present criteria. It seems safe at the moment, and even advisable, to postpone the decision regarding the designation of a lectotype for this species, which differs from the above-described taxa with self-capitate, bulbed cystidia, by the possession of larger, conical-stemmed cystidia and slender, cylindrical spores.

PENIOPHORA JUNIPERINA var. SORORIA (Bourd. & Galz.) Bourdot & Galzin, Hym. de Fr. p. 290 ([1928])

P. sororia Bourd. & Galz., Bull. soc. mycol. France, **28**, 386 ([1913]) (not *P. sororia* G. H. Cunn., Trans. Roy. Soc. N.Z. **83**, 280 (1955))

P. glebulosa ssp. *sororia* (Bourd. & Galz.) Pilát, Bull. soc. mycol. France, **42**, 115 (1926)

No authentic material of this taxon has been available to me. And Bourdot judged it, at least eventually, as so close to *juniperina* that it merited no more than varietal status. Should the examination of syntypic material prove him wrong—the original description suggests *P. accedens* rather than *P. juniperina*—it may be necessary for the name *P. sororia* to supplant the name *P. accedens*. But such considerations must await the appearance of syntypic material.

Peniophora juniperina var. *subquercina* Donk, Mededel. Ned. Mycol. Ver. **18–20**, 166 (1931)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN M.A.D.
(on bark of fallen branch of *Quercus robur;* Bilthoven (Ut.); X.1929; M. A. Donk, *1816*)

FRUCTIFICATION white, from delicate thin meshwork to dense reticulum with occasional slight papillation; more or less separable. CONTEXT composed of nodose-septate hyphae, to 3 μ in diameter, with firm walls. CYSTIDIA self-capitate, with subconical stems, 30–65 [Donk: to 75 μ] × (3)4.5–6.5 μ just above the usually furcate base, sometimes distinctly truly bi-rooted; narrowing more or less uniformly to a neck width of (2)3–3.8(5) μ; wall varying in amyloidity from negative to strongly positive, smooth, thick, middle layer soluble in 10% KOH, not colored by Congo red; lumen capillary in base and stem, expanding abruptly at neck to form thin-walled apical bulbs (4)9–11 × (4)8.5–10 μ; sometimes collared by crystals, sometimes haloed or capped by an amorphous or crystalline mass. Occasional cystidia subconical and lacking bulb, lumen not capillary except in roots or not even there, relatively broad in stem, with wall thinning gradually to elongate, thin-walled apices. BASIDIA flexuous cylindrical to clavate to obovoid, usually narrowing sharply below, ca. 15 × 4.3 μ (few measured), tetrasterigmate. BASIDIOSPORES (few seen) cylindrical, curved, 5.5 × 1.5 μ [Donk: 5–6 × 1.5–1.75–2 μ]; wall thin, smooth, non-amyloid.

Although little attention is given here to infraspecific taxa, this description is included because it provides, for comparison, a description of what I consider to be more or less typical *P. juniperina*, in spite of its occurrence on oak instead of conifer or heath. The name is therefore placed in synonymy, although it may eventually be possible to distinguish it at the varietal level from the type variety.

TUBULICRINIS BOREALIS J. Eriksson, Symbolae Botan. Upsaliensis, **16**(1), 79 (1958) (TYPE: Olsson & Eriksson *7291*) (n.v.)

This name is recorded here for the sake of giving as complete a record as possible of the taxa known to be related to the species of Tubuliferae as conceived by Bourdot and Galzin. The description given by Eriksson places it close to *P. juniperina*, from which it appears to be distinguishable mainly by the greater breadth of its spores.

PENIOPHORA REGIFICA Jackson & Dearden, Mycologia, **43**, 57 (1951)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(on coniferous wood; Peavy Arboretum, Benton Co., Oregon; 11.XI.1940; M. Doty *5236*)

FRUCTIFICATION white, drying to a crustose film, more or less adherent; surface tufted and pronouncedly hispid; margin of crust abrupt, but with an outlying sprinkling of cystidia lacking an observable subiculum. CONTEXT as much as 170 μ deep; composed of nodose-septate hyphae, 2.8–4.3 μ broad, with thin to firm walls, ascending from a narrow basal layer; apparently embedded in a matrix; all tissue except the cystidia so easily pulped that structures collapsed and indistinct. CYSTIDIA more or less embedded or, most frequently, emergent to as much as three-quarters of height; self-capitate, cylindrical to subconical in the stem, up to 150 μ long, (5.5)7–8.8(10) μ wide just above the furcate base, truly bi- to several-rooted; typically narrowing to a neck diameter of 3.5–7 μ; wall non-amyloid, smooth, thick, middle layer soluble in 10% KOH, not colored by Congo red, thin outer and inner membranes insoluble, staining in Congo red; lumen capillary in base and stem, expanding more or less abruptly at neck to form thin-walled apical bulbs, as much as 18 × 13 μ [Jackson & Dearden: 10–12 μ broad] (one seen 16 μ in width); sometimes haloed by amorphous mass or lightly encrusted. Occasional cystidia subcylindrical or subconical throughout, lacking bulb and with lumen capillary only in base and lower stem, expanding gradually into elongate clavate or cylindrical apices; rare buried cystidia with stems abruptly attenuated into elongations of just firmly outlined lumina. BASIDIA broadly clavate or flexuously cylindrical, 18–22 × 6–7.5 μ; frequently obscured by generalized pulping of tissue, apparently with clamp at narrowed base whether terminating a basidiophore or sessile on a repent hypha; with four slender erect sterigmata as much as 8 μ long. BASIDIOSPORES broadly ellipsoid, 7–8.5 × 4–5.5 μ, adaxially flattened; wall thin, smooth, non-amyloid.

PENIOPHORA HAMATA Jackson, Can. J. Research, C, **26**, 133 (1948)

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN TRTC, DAOM
(on *Abies balsamea*, Timagami, Ontario; 12.IX.36; R. Biggs. TRTC *9871*)

FRUCTIFICATION creamy white, unevenly thickened into dense, smooth pads separated by a coarsely porose reticulum; hispid in profile; separable. CONTEXT tissue lax, mainly collapsed, composed of thin-walled, nodose-septate hyphae, variable in diameter from 2.8 to 5 μ. CYSTIDIA with obclavate stems capped by caducous convex parasols of composition different from that of the cystidial walls; in two size-ranges, the miniature (perhaps juvenile) with stem up to 30 μ tall, swollen at the base to a diameter of 3.5–6 μ, narrowing upward to a tip 1.5–2 μ wide and topped by a cap ca. 4 μ deep and 5 μ broad; the mature with stem up to 60 μ tall, swollen at the base to a diameter of 10–15 μ, narrowing to a neck ca. 3.5 μ wide and broadening slightly (to perhaps 4 μ wide) at the tip (exposed only when cap falls off), parasol as much as 7 μ deep, and up to 10 μ broad, bearing 9–18 marginal down-curved prongs ca. 3 μ long; base with occasional demonstration of hyphal attachment, rarely a true bi-rooting; wall thickened somewhat, but rarely to as much as 2–3 μ, and to this extent only medially and at the extreme tip of some cystidia, non-amyloid, the middle lamella of the wall relatively promptly soluble in 10% KOH, outer and inner membranes less sensitive to alkali and colored by Congo red; the caps neither staining in Congo red nor promptly soluble in KOH. BASIDIA mainly collapsed, clavate, up to 30×8.5 μ (at widest), with 2–4 fleshy sterigmata as much as 8.5 μ long. BASIDIOSPORES broadly ellipsoid, 5.5–7(8.2) × 4–5.5 μ; somewhat flattened adaxially with prominent lateral apiculus; wall thin, smooth, non-amyloid.

PENIOPHORA UMBRACULA Cunningham, Trans. Roy. Soc. N.Z. **83**, 291 (1955)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(on *Pseudowintera colorata;* Stewart Is., N.Z.; Feb. 1954; J. M. Dingley; P.D.D. herb. *13660*)

FRUCTIFICATION white to creamy, from a thin but coarsely porose reticulum to a dense, submembranous pad; pronouncedly hispid; more or less adherent to substrate. CONTEXT tissue lax, composed of thin- to firm-walled, nodose-septate hyphae, variable in diameter 2.8–5 μ. CYSTIDIA with obclavate stems capped by caducous convex parasols of composition

different from that of the cystidial walls; ranging in size from the miniature (juvenile) with stems up to 35 μ tall, swollen at the base to a diameter of 3–6 μ, narrowing to a tip 1–2 μ wide and topped by a cap ca. 3 μ deep and 4.5 μ broad—to the mature with stem up to 80 μ tall, swollen at the base to a diameter 7.5–14 μ, narrowing to a neck ca. 3.5–7 μ wide, tapering further to a tip as little as 3 μ wide (exposed only when cap falls off), parasol as much as 7 μ deep and up to 8 μ broad, bearing up to about a dozen marginal down-curved prongs; base sometimes narrowing sharply below vesicle into stalk and basal clamp, sometimes vesicle sessile on true or false bi-rooting; wall thickened more or less, frequently one-sidedly to as much as 2–3 μ, the middle lamella of the wall relatively promptly soluble in 10% KOH, outer and inner membranes less sensitive to alkali and colored by Congo red; the caps neither staining in Congo red nor promptly soluble in KOH. BASIDIA flexuous cylindrical to subclavate to ovoid, up to 20×6.5 μ (at widest, either midway or toward the apex); with clamp at base; tetrasterigmate. BASIDIOSPORES ovate-ellipsoid to oblong, adaxially flattened, 5–6 × 3–3.5 μ; wall thin, smooth, non-amyloid.

P. umbracula from New Zealand and *P. hamata* from Ontario (cf. above) are so distinctive and so close that one hesitates to consider them separate species. However, the type specimens are certainly distinguishable on the basis of the differences pointed out by Cunningham (*loc. cit.*), particularly the spore shape, for the spore of *umbracula* is more elongate in relation to its width, with a tendency toward ovateness lacking in the broadly ellipsoid spore of *P. hamata*. The possibility that they are distinct only at the varietal level cannot be dealt with at this time.

PENIOPHORA CRASSA Burt ex Peck, N.Y. State Museum Rept. **54**, 155 (1901)

Peck's original description is based on his own collection on "decorticated surface of trunks of pine, Floodwood, August". It is this specimen which must be taken as type of the species, in spite of Burt's later ([1926]) designation of a Vermont specimen. From the Burt herb. at FH came a collection marked Peck No. 2, with the requisite collection data, undoubtedly that referred to by Peck.

DESCRIPTION OF PORTION OF TYPE COLLECTION IN FH
(on pine; Floodwood, N.Y.; August 1900; C. H. Peck *2*)[6]

FRUCTIFICATION effused; pale cream to rosy or yellowish cream, cushiony fibrous; in age or when wounded, hymenial surface browned and vernicose-waxy; surface velvety, "smooth when collected, cracked in drying" [quotation from Peck, in Burt's hand], in patches villose with uncrowded emergences of pseudocystidial apices, in other patches these apparently flattened or destroyed; cracks like craters and interconnecting valleys, ripping the fibrous context down to the substrate; more or less completely separable; margin strongly furry fibrous, radiating, determinate. CONTEXT composed of a more or less horizontal subiculum of intertwined hyphae, with a deep (as much as 500 μ and more) upper palisade of hyphae and pseudocystidia; clamp connections prominent, sometimes somewhat "ansiform"; hyphae 2–3.5–4.8 μ in diameter, with walls emphasized or thickened, in many (as seen in lactophenol or in Melzer's) to the point of capillarity of the lumen, especially in lower parts of the fructification, progressively thinner-walled toward the hymenium; layers of wall sensitive enough to alkali to make measurements in such media unreliable, though a substantial width apparently left undissolved, colored by Congo red; pockets of hyphal knots present occasionally in some parts of context, formed by short, stumpy branches arising in groups from a clamp and twisting around the original hypha. PSEUDOCYSTIDIA embedded or projecting as much as 80 μ; very long, often extending deep into the subiculum, with basal septum obscured; usually more or less clavate, with thick stem walls, non-amyloid, extremely sensitive to alkali, dissolving except for thin inner membrane which stains in Congo red; lumen expanding more or less gradually toward obtuse, often collapsed apex, sometimes sprinkled or engrained with scattered crystals or massed with conglutinated crystals-and-spores as a cap or series of halters; 7–8.5(12) μ at broadest; cystidial stems so brittle that they are usually observable only as segments with thick walls and capillary lumen, and that only in acid medium. BASIDIA very slender, no more than 2 μ at the apex, broadening below tip to a cylinder no more than 3 μ broad, rising above the hymenial surface when mature, with four spidery sterigmata. BASIDIO-

[6]NOTE ADDED IN PROOF: A collection of *Peniophora crassa* Burt (labelled "Coll. N.Y. State; on pine; Floodwood; Aug.; Chas. H. Peck"), deposited in the Peck herb. at the N.Y. State Museum in Albany, was received when this paper was in press. The specimen appears to be a portion of the collection here designated as type, but is in poorer condition and provides no additional information.

SPORES allantoid, with highly refractive, heterogeneous cytoplasm when first mounted, typically in tight orange-section clumps at apex of basidium or when floating free; 5–6 × 1.5–1.8 μ; wall thin, smooth, non-amyloid.

As has already been pointed out (Eriksson 1958; Weresub 1957), the "lyocystidia" of *P. crassa* are of quite different nature from those in *Tubulicrinis*, and several other differences make it unlikely that this species belongs in that genus. However, this is a matter for discussion elsewhere.

Peniophora verticillata Burt, Ann. Missouri Botan. Garden, **12**, 285 ([1926])

DESCRIPTION OF PORTION OF TYPE COLLECTION IN BPI
(on rotten coniferous wood; Waltersville, Oregon; March 20, 1922; C. C. Epling & J. B. Shorett, *600*; Comm. S. M. Zeller *2317*; MO *63041*)

FRUCTIFICATION mainly pinkish buff; surface smooth, hispid; up to ca. 1.5 mm thick; margin petering out into fibrous radiations. CONTEXT compactly fibrous, basally of intertwined hyphae, with a deep upper palisade of hyphae and cystidial stems; clamp connections prominent; hyphae with wall thin or thickened, sometimes to the point of capillarity of lumen; middle layer sensitive to KOH; hyphae pronouncedly inflated at points of dichotomous branching, with frequent and prominent anastomoses. PSEUDOCYSTIDIA embedded or emergent to 30 μ [Burt: to 45 μ]; very long [Burt: 150–200 × 6–7 μ], often extending deep into the subiculum, basal septum obscured; usually more or less clavate, with thick stem walls, non-amyloid, dissolving in KOH; lumen expanding more or less gradually toward obtuse, often collapsed apex, with or without engraining or deep halters of crystals; cystidial stems variable in diameter, up to ca. 6.5 μ, so brittle that they are usually observed only as segments with capillary lumen, rapidly dissolved in KOH. No mature basidia seen, but basidioles slender, ca. 2 μ in diameter. BASIDIOSPORES often in clumps of four; allantoid, typically narrowed toward the apiculate end; 5–7 × 1.3–1.8 μ [H. S. Jackson: 4.5–5.5 × 1–1.5 μ]; wall thin, smooth, non-amyloid.

As is seen from this description, Rogers and Jackson (1943) were completely justified in placing this taxon in synonymy with *P. crassa*.

Stereum karstenii Bresadola, I.R. Accad. Agiati Atti **111**(3), 108 (1897) (not *P. karstenii* Massée, J. Linnaean Soc. **25**, 153 (1889))

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN FH
(in truncis Abietis pectinatae; Hungary: Kmet; in herb. Burt ex Bres.)

FRUCTIFICATION buff when young, in age avellaneous, rather spotty; surface when young smooth pruinose, in age furry; up to 1 mm or more in depth; margin furry but clearly delimited. CONTEXT compactly fibrous, basally of intertwined hyphae, many browned by resinous deposition in cytoplasm, with deep upper palisade of hyphae and cystidial stems; distinctive clamps prominent when hyphae adequately separated; hyphae 2.5–4.5 μ broad, with walls emphasized or thickened sometimes to the point of capillarity of the lumen; middle layer sensitive to KOH, but outer membrane, usually colorable by Congo red, sometimes firm enough to maintain shape of hypha intact after KOH action; crystals scattered or massed throughout. PSEUDOCYSTIDIA embedded or projecting, very long [Burt: perhaps 200 μ long, 6–8 μ thick], often extending deep into the subiculum, with basal septum obscured; usually more or less clavate, with thick stem walls, non-amyloid, dissolving in KOH except for the sometimes visible outer membrane which stains in Congo red; lumen expanding more or less gradually toward obtuse, often collapsed apex, up to 8.5 μ broad, sprinkled with crystals or naked; cystidial stems variable in diameter, up to ca. 7 μ, so brittle that they are usually observed only as segments with capillary lumen, rapidly dissolved in KOH. No mature BASIDIA seen, but basidioles no more than 2 μ broad at apex, swelling below to 2.5 μ. BASIDIOSPORES often in clumps of four; allantoid, typically narrowed toward apiculate end; 4.5–5.5 × 1.2–1.8 μ; wall thin, smooth, non-amyloid.

As the descriptions of the type collections demonstrate, *Stereum karstenii* and *Peniophora crassa* are one species. They were placed in synonymy by Burt under the latter name because the former epithet was preoccupied in the genus *Peniophora* by a Massée name. So long as it remains in *Peniophora*, the taxon must be called *P. crassa*, and be typified by the *crassa* type specimen, but when transferred to another genus (e.g., cf. Donk 1956*b*: *Tubulicrinis karstenii*) in which the epithet *karstenii* has not been used, it will reclaim

that epithet and the corresponding type specimen here described, discarding the later replacement.

STEREUM KARSTENII f. INCRUSTATA Pilát, Bull. trim. soc. mycol. France, **49**, 44 (1933) (n.v.)

Pilát distinguished this form as having numerous cystidia which become encrusted, his illustration showing these encrustations as a series of halters along the emergent length of the cystidium. This is exactly the arrangement of the encrustations on the pseudocystidia of *P. verticillata*. But Pilát gives the basidia as 3–5 μ in diameter, and the spores as elliptic, 4–4.5 $\times$ 2–2.3 μ. These widths are considerably greater than those of the basidia and spores of *P. crassa* (cf. Pilát's own description, [1930] p. 100, of *S. karstenii*, as well as descriptions of the type specimens here). Re-examination of the material of this 'form' will be necessary before its place in the synonymy of this species (cf. Rogers and Jackson 1943) is assured.

Peniophora farinacea Bourdot & Galzin, Hym. de Fr. p. 292 ([1928])

This species has no history beyond its description, based on a specimen cited as "Mai. Sur bois denudé des branches tombées de pin, Labastide-Pradines (Aveyron)". What is probably the only known specimen (and therefore type) is a collection labelled in Bourdot's hand (in PC): "Bourd. *9356¢*; Champs. de l'Aveyron; *13299* des récoltes de M. Galzin; Envoi du 13.V.1913; Labastide-Pradines; *P. farinacea*".

DESCRIPTION OF PORTION OF TYPE COLLECTION IN PC
(Bourd. *9356¢*, Galz. *13299*)

The various pieces of wood that make up this collection bear patches of fungal growth that are rather diverse in gross appearance. A patch that answers to the description "farineux, subpapilleux, puis finement glébuleux" yields a mount that approximates the Bourdot illustrations both on the packet of this collection and in print. A few long cystidiform hyphae, thick-walled with capillary lumen, are scattered about on the substrate, some perhaps acting as cystidiophores. A few cystidia are about, mainly embedded in formless tissue; their stems are largely cylindrical, about 6–7.5 μ wide, with thick walls of doubtful amyloidity; their thin-walled apices are generally lacking or more or less collapsed, but a few fairly elongate, cylindrical apices show up (13$\times$5 μ). Some basidial husks give a suggestion of structure to the amorphous mass of tissue. Over this mass, as illustrated by Bourdot, is a layer of fruiting material. This is composed of generally horizontal hyphae, thin-walled and staining in Congo red, somewhat irregular in diameter with a suggestion of swelling at the approach to a clamped septum. Arising perpendicular to these hyphae are cylindrical to subclavate or obovate basidia, largely 10–15 $\times$ 3–4.3 μ, bearing spores with pronounced walls staining heavily in Congo red, broadly obovoid to subglobose in one view, ellipsoid with a flattened side in the other view, with a prominent apiculus; 3–4 $\times$ 2.5–3.3 μ. There are also a few slender, more or less fusiform cystidioles present, and the occasional slender cylindrical spore, 5–5.5 $\times$ 1.8–2 μ.

There appears to be no connection except a spatial one between the fruiting layer with its subglobose spores and the main mass of formless tissue with cystidia and slender spores. Apparently the latter belong to what may have been *P. gracillima*, overgrown by what is likely to be, from the appearance of the hyphae, basidia, spores, and cystidioles, *Corticium confine* Bourd. & Galz.

P. farinacea, therefore, is judged to be a ***nomen confusum***.

Peniophora gilva Bourdot & Galzin, Hym. de Fr. p. 284 ([1928])

The single citation in description states: "Septembre; sur sapin; Turini, Alpes Mar. (E. Gilbert)". The packet label of the collection at PC is written in Bourdot's hand: "Des récoltes de M. Gilbert dans les Alpes maritimes, IX.1925; Bois de sapins, Turini; G. *1148; ¢39441; P. gilva* mihi n.sp.". This is clearly the type and only specimen of *P. gilva*.

Examination of the material yields the following observations. Part of the material is delicately arachnoid subpellicular and in poor condition, possibly *Pellicularia vaga* (Berk. & Curt.) Rogers. The subceraceous portions described by Bourdot are flecked with resinous specks visible under the dissecting microscope; and the microstructures confirm the evidence of gross appearance that this is an extremely thin form of *Corticium pallidum* Bres.

The name *P. gilva*, therefore, may be thrown out as a case of mistaken identity, a synonym of *C. pallidum*.

Hyphodontia J. Eriksson 1958

The Tubuliferae of close affinity to *Peniophora subalutacea* have been included in this genus by Eriksson (1958), and are grouped here under that name for convenience. It may be pointed out that, in the type species, *P. pallidula* (Bres.) Bres., as we know it here (cf. also Boidin 1958*b*, p. 261), the walls of all structures (except spores) are typically more or less strongly pseudamyloid (i.e. brown in Melzer's—cf. Singer [1951]); whereas there is no more than a generalized yellowing of tissue in Melzer's in members of the *subalutacea*-group. However, further discussion on relationships is better withheld for presentation at a later date.

ODONTIA ABIETICOLA (Bourd. & Galz.) Lund., *in* Lundell and Nannfeldt, Fungi Exs. Suecici, No. 738 (1939)

O. barba-jovis ssp. *abieticola* Bourd. & Galz., Hym. de Fr. p. 426 ([1928])

As is seen from its generic category, this taxon was not included by Bourdot and Galzin in their Tubuliferae. However, because its close relationship to *P. subalutacea* has been pointed out by them as well as by Eriksson (1958), and its possible identity with *P. alienata* by Weresub (1957), it seems advisable to treat it here in order to fix its type.

Bourdot and Galzin described this subspecies of *O. barba-jovis* on the basis of collections cited as follows: "Été, automne. Sur bois des souches très pourries de sapin pectiné, Vosges: Corcieux, Vanémont; Gérardmer (L. Maire); Alpes mar.: Turini (E. Gilbert)".

From PC came three collections: Bourd. *4141A¢* ("Champs. des Vosges, sur sapin, Corcieux, Galz. *409*; Nov. 8, 1904"); Bourd. *39487* ("=*39469*; Champs. des Alp. mar., sapin, Turini, E. Gilbert *1194*; IX. 1925"); and Bourd. *25001* ("Champs. des Vosges, sur sapin, Gérardmer, L. Maire *560*; Sept. 1918").

Bourd. *25001* (Maire *560*) proved to be *O. lactea*; and this explains Bourdot's statement: "quand elles [the cystidia] font entièrement défaut, la plante devient très voisine de *O. lactea*". The other two collections appear to account for the other citations, except for the Vanémont specimen.

These three specimens were the Bourdot material available to me at the time of the writing of my thesis, when I stated that, because the two (Bourd. *4141A¢* and *39487*) which fit the original description adequately seem to be indistinguishable from *P. alienata*, it would be necessary to place the two names in synonymy. At the same time, reference was made to the Lundell collection *1480* (Fungi Exs. Suec. *738*) as possibly different.

The last-named collection was what Eriksson (1958) used to illustrate

Hyphodontia abieticola (Bourd. & Galz.) Erikss. He and I were using the same name, but for what are apparently different taxa. Fortunately, I have since received from Dr. Donk another syntypic specimen, Bourd. *24478* ("Ch. des Vosges, *Odontia abieticola* p.t., sur sapin, Gérardmer, L. Maire *492*, VI.1918"). It also fits the original description; and the existence of this collection may make it possible to retain *O. abieticola* in the sense in which it has been used by such European authors as Christiansen (1953, 1960), Eriksson (1958), and Lundell (*loc. cit.*). It is therefore this specimen which is here chosen as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN M.A.D. (*2408*)
(Bourd. *24478*, Maire *492*)

FRUCTIFICATION ochraceous to yellowing to brown-glazed where pressed or aged; more or less broadly effused (difficult to tell because substrate so badly rotted, stringy and uneven); reticulum delicate in younger patches, coarsely poroid where older; occasional irregularly scattered, threadlike teeth present, in addition to a more general villosity; margin apparently indeterminate. CONTEXT of distinct hyphae, firm-walled, prominently clamped at every septum, with wide-angled branching; 2.8–4.5 μ in diameter, with wall up to 0.8 μ wide; in the subiculum with longer cells, somewhat thicker walls than in the trama, but not pronouncedly different. Teeth composed of compact clumps of cystidia, surrounding or associated with a core of straight, sparingly branched 'extension' hyphae, firm-walled and long-celled, as well as an upgrowth of subhymenial and hymenial elements around this axis. CYSTIDIA in sheaves of half-a-dozen or more, or loosely distributed singly or in small-numbered groups, variable in length, depending on age and position in tooth or smooth parts of fructification, as much as 200 μ long, some emergent almost their total length but the oldest originating well in the depth of the context, frequently with a trailing hyphal cystidiophore; subcylindrical to subclavate, apical terminations to the cystidiophores, usually narrowing rather sharply to the basal septum —3.5–4.5 μ in width there—enlarging upward to a width of 6–7(8) μ in the stem, 4–7.5 μ at the apex which is generally obtuse though somewhat narrowed; false septa frequent across the lumen; wall thickened except at apex, usually broadest at the greatest width of the cystidium, there as much as 1.8(–2.5) μ broad, staining throughout in Congo red, non-amyloid. BASIDIA mainly collapsed and therefore difficult to study, but apparently subclavate or clavate at maturity, sinuate or straight, up to 25 μ in length, 5.5–6.5 μ at the broadest point. BASIDIOSPORES oblong or broadly ellipsoid to broadly ovate, 6–7 $\times$ 3.5–4.2 μ; wall thin, smooth, non-amyloid.

PENIOPHORA ALIENATA Lund., *in* Lundell and Nannfeldt, Fungi Exs. Suecici, No. 1043 (1941)

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN TRTC, NY, AND DAOM
(on decaying prostrate trunk of *Alnus glutinosa;* Göteborg, St. Anggården, Sweden; 5.VI.1940; T. Nathorst-Windahl *1950*; F.E.S. *1043*)

FRUCTIFICATION pale cream to a clear yellow in color, broadly effused, soft, densely reticulate, uniformly villose, flocculose, with irregular clumping, almost mounding of the surface; separable; margin indeterminate. CONTEXT composed of a few repent, subicular hyphae, long-celled (up to ca. 170 μ long), rarely branching to give off erect stalks from which tramal tissue develops; this made up of intertwined, frequently collapsed hyphae, thin- to firm-walled, prominently clamped at every septum, with wide- to right-angled branching; 3.5–5(6.5) μ in width; progressively shorter-celled toward the hymenium. CYSTIDIA sometimes grouped but generally more or less uniformly distributed; emergent as much as 85 μ, the emergent portion massed or sprinkled with blocklike crystals or chips, easily dislodged; subcylindrical apical terminations to cystidiophores, as much as 130 μ in length; (3)5–8 μ wide at the basal clamp, 4–8–9.5 μ at the broadest part of the stem [Lundell: 4–6 μ wide, 8–9 μ with encrustation], generally narrowing somewhat to an obtuse or tapering apex that is 4.5–6.3 μ wide; wall thickened at least in lower half, sometimes almost to the apex, usually broadest at the greatest width of the cystidium, there as much as 1.5–2 μ wide, staining throughout in Congo red, non-amyloid. BASIDIA sinuately cylindrical, 15–20 $\times$ 4–5 μ; four sterigmata erect, slender, 3.5 μ long at maturity. BASIDIOSPORES oblong-ellipsoid to approaching ovate, 5–6–7.2 $\times$ (2.8)3–3.8 μ; wall thin, smooth, non-amyloid.

There are several differences between the now-designated lectotype of *Odontia abieticola* and the above-described type collection of *P. alienata:* a softer, more cushiony fructification in the latter, lacking the coarseness and

occasional toothing of the former; the presence of vertical 'extension' hyphae in *O. abieticola* which make its teeth more than simple clumps of cystidia, the more uniform diameter of its more consistently firm-walled hyphae, the more frequent occurrence of an abrupt narrowing to the basal septum of its cystidia which are also longer, their walls slightly thicker and thickened for a greater portion of the length of the cystidium; and finally, the somewhat greater width, in relation to length, of the basidiospores of *O. abieticola*. Since the types of these two taxa are clearly distinguishable, there can be no question of synonymy until consideration is given to the characters of large numbers of collections assigned to the two. Unfortunately we do not have interfertility tests to aid us, and the decision will be made purely on a morphological basis when specific descriptions are given.

PENIOPHORA SUBALUTACEA (Karst.) Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **115**(I), 1601 (1906)

Corticium subalutaceum Karst., Soc. Fauna & Flora Fenn. Medd. **10**, 65 (1883)

The only Karsten specimen available to me is that referred to by Burt ([1926]) as "perhaps part of type".

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN FH
(Mustiala, in ligno Pini, P. A. Karsten)

FRUCTIFICATION cream buff and pinkish buff, to yellowish, with aging or bruised areas browned; a widespread, more or less woolly network, tufted in some areas and hispid with cystidia, completely smooth in others; margin indeterminate, more or less distinctly meshlike; separable, with attached surface slightly darker than hymenial. CONTEXT of loosely woven, distinct, mainly erect hyphae, 2.5–4 μ in width, with clamp connections at all primary septa, some hyphae with simple 'secondary' septa; branching frequently right-angled, often with perpendicular upturning of a branch to run parallel to the original hypha; wall generally thin on subhymenial hyphae, thicker (up to 0.5 μ in width) on subicular hyphae; smooth, non-amyloid, strongly stained by Congo red. CYSTIDIA immersed or emergent up to 25 μ (up to 50 μ in the midst of a hymenial clump), long subclavate, as much as 250 μ long, 3–4.5 μ broad at the base, 6.8–9 μ wide at the broadest part of the stem, 6–7.5 μ broad at the apex; wall smooth, non-encrusted, thickened as much as 1.5–2.5 μ in the stem and base, thinning toward the apex, non-amyloid, staining strongly in Congo red, apparently unaffected by KOH; occasionally secondarily simple-septate; originating at a clamp as modified ends of erect hyphae, the most mature from near the substrate, younger cystidia appearing in various stages of development throughout subiculum and subhymenium, extending into and usually slightly above the hymenium. BASIDIA clavate to cylindrical, straight to somewhat flexuous, 15–25 $\times$ 3.5–5.5 μ at the apex, with four sterigmata that are fairly fleshy below but soon drawn into arched threads up to 7.5 μ long. BASIDIOSPORES cylindrical, curved in profile, often particularly so toward the apiculus; 6.5–8.5(9) $\times$ 1.8–2(2.5) μ; wall thin, smooth, non-amyloid.

PENIOPHORA SUBALUTACEA f. CASTANEAE Bourdot & Galzin, Hym. de Fr. p. 293 ([1928])

It is only because a collection of this 'form' has come to hand that the taxon receives consideration here. The specimen is in both S and UPS: Bourd. *32012*, Coll. Galzin *14153*, 13.XI.1913, "sur châtaignier, Bordes, Aveyron". It is undoubtedly syntypic, but I hesitate to assign it the status of lectotype, because it does not illustrate, to any marked degree, the distinguishing character reported by Bourdot, namely: "cystides fasciculées". The label notes that this number "$\doteq$*13744* G.". It seems advisable to let choice of a lectotype await examination of Galzin's collection No. *13744*, which may fit the description more nearly.

PENIOPHORA SUBALUTACEA ssp. CINERACEA (Bourd. & Galz.) Bourdot & Galzin, Hym. de Fr. p. 293 ([1928])

P. glebulosa ssp. *cineracea* Bourd. & Galz., Bull. soc. mycol. France, **28**, 387 ([1913])

A "nov. subsp." in "slanted type" in the 1913 publication, this taxon was placed at the end of a list of several taxa apparently of the same rank, following *P. glebulosa* and immediately preceding *P. subalutacea*. A footnote to the description compares it, not with *P. glebulosa*, but with *P. subalutacea*. Saccardo (1925, p. 536) marked it "subsp.", adding "Pen. subalutaceae et Pen. glebulosae affinis". It was, it seems, to be considered a step between the two, and whether a subspecies of one or of the other did not matter, since the 1928 "Hyménomycètes" shows its name again in the "slanted type" of subspecific rank, but this time below *P. subalutacea*.

The 1913 citation gives the following data: "Aestate; ad Ericam arboream, Aveyron". Two authentic collections have been available in this study: from herbs. S and M.A.D. (*2734*), Bourd. *32017* ("Galzin *13427*, 9.VI.1913; ad ramos Callunae vulg., Balzaguet, Aveyron"); and from herb. M.A.D. (*2732*), Bourd. *8015* ("Galzin *6731*, 9.VII.1910, sur *Erica arborea*, Aveyron"). The latter collection seems to have been the only one Bourdot had at hand when he described the taxon. Dr. Donk has justifiably labelled his portion of it "part of type", and it is so designated here.

DESCRIPTION OF PART OF TYPE COLLECTION IN M.A.D. (*2732*)
(Bourd. *8015*, Galz. *6731*)

FRUCTIFICATION an interrupted reticulum, uniform and pale cream where young, strongly interrupted to the point of porosity and yellowing where older; browning in age especially where poroid appearance emphasized by dehydration causing withdrawal or disappearance of most of the hyphae from between 'walls' of 'pores' of mesh; separable; margin indeterminate. Condition rather poor; much of tissue collapsed, inhabited or overgrown by hyphomycete, but characterization possible. CONTEXT of loosely interwoven, distinct, mainly erect hyphae, nodose-septate, uniform in diameter, 2.8–4 μ wide; wall generally pronounced to somewhat thickened, colored by Congo red. CYSTIDIA emergent half-way or more; generally apical terminations of cystidiophores, sometimes with suggestion of basal furcation, usually false; more or less cylindrical, typically tapering sharply below, sometimes with trailing root; narrowing also above, but only slightly, to rounded apex; occasionally secondarily septate; length up to or exceeding 165 μ, width 8–11.5 μ at the widest part of stem (approximately midway), narrowing below to 4–5.5 μ at the basal septum, and above to 6.5–7.5 μ at apex; wall smooth, non-encrusted, thickened to as much as 3.5 μ at the broadest part of stem, narrowing gradually toward thin-walled apex, non-amyloid, staining strongly in Congo red, little affected by 10% KOH. BASIDIA mainly collapsed at maturity so that few reliable measurements available; apparently clavate, becoming more or less truncate at maturity, measuring ca. 20×6 μ; tetrasterigmate. BASIDIOSPORES few, cylindrical, straight to curved, 6.5–7.5 × 2–2.5 μ; wall thin, smooth, non-amyloid.

The affinity of this taxon to *P. subalutacea* is evident; but its maintenance at the subspecific level is questionable. When the only characteristics available are morphological, and two taxa appear to be distinguishable on the basis of these characters, it becomes necessary to specify how many or what kind of distinctions are sufficient for subspecific delimitation, and how many or what kind for designation at the specific level. What usually happens instead is that the taxonomist gives an instinctive evaluation of the differences, without explaining why these have greater or lesser significance than the differences between other taxa in the same genus. Although the instinctive decision is often right, unless it is backed by communicable evidence it is too authoritarian to be acceptable. It seems to me, therefore, that, in the absence of

contradicting evidence from cultures and mating tests (cf. Weresub and Gibson 1960), two distinguishable taxa should be regarded as different species.

The comparison between the lectotype of *P. subalutacea* and the type material of its "subspecies" *cineracea* shows differences between the two in choice of host, in gross appearance of their fructifications, in emergence of cystidia, and in furcation of their bases, and especially in the ratio of length to width of their basidiospores. If these differences are maintained when more material of both taxa is examined, it will be necessary to establish *cineracea* as a separate species.

PENIOPHORA GLEBULOSA ssp. CINERACEA var. MINOR Pilát, Bull. soc. mycol. France, **42**, 115 (1926) (n.v.)

Until material of this variety is re-examined for characters now used in distinguishing and relating the taxa of these groups, no useful attempt can be made to evaluate its status as a variety of *cineracea*. It seems to belong to the *P. subalutacea* group, as does the subspecies within which it has been placed, rather than to the "*P. glebulosa* group" in *Tubulicrinis*. But its classification at the varietal level may be questioned if the principle outlined above is adhered to, since the var. *minor* differs from *cineracea* at least in the possession of smaller, narrower cystidia, broader spores, and in host preference. However, no decision can be made here.

PENIOPHORA SUBALUTACEA ssp. LURIDA Bourdot & Galzin, Hym. de Fr. p. 294 ([1928])

The taxon is described as based on a specimen cited as "Autumne. Sur genévrier, Aveyron". The only collection available during this study is quite likely to have been the only specimen Bourdot had at hand: "Bourd. *9180¢*; Champs. de l'Aveyron; *12063* des recoltes de M. Galzin; Envoi du 13.XI.1912; sur genévrier; Vignoles". It appears justifiable to consider this collection the type specimen of this taxon.

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN PC AND M.A.D. (*2733*) (Bourd. *9180¢*, Galz. *12063*)

FRUCTIFICATION light pinkish cinnamon to pinkish cinnamon; soft-cushiony, dense, farinose-crumbly; separable. CONTEXT of coarse hyphae arranged in palisade, generally 4–5 μ wide with wall thickened as much as 0.7–1 μ, hyphal diameter sometimes swollen to as much as 9 μ, with wall up to 2 μ wide; large clamps at every septum. CYSTIDIA emergent to 60 μ; frequently with minute crystals and amorphous material clinging to apex, easily dislodged and apparently soluble in alkali; subconical in shape, broad at the base and furcate, or subfusoid, arising directly from a more or less erect cystidiophore; 40–80–100 × 4.5–8–12 μ broad just above the fork, 6–10 μ broad in the stem, and generally tapering upward to a somewhat cylindrical to conical apex 2.7–5.5 μ wide, thinner-walled; sometimes secondarily septate across lumen; wall more or less uniformly thickened, 0.7–1.3 μ in width, non-amyloid, staining throughout in Congo red, apparently unaffected by 10% KOH. BASIDIA produced in candelabrum arrangement, 20–24 × 4.7–6 μ, with four erect sterigmata. BASIDIOSPORES variable in shape: in face view, consistently ellipsoid, narrowing to the prominent apiculus; in profile or half-profile view, uniformly ellipsoid, or ovate, or oblong to ellipsoid with adaxially flattened side; 5–7 × 2.5–3.8 μ; some apparently engrained with or containing a resinous material; wall thin, smooth, non-amyloid.

Comparison between this specimen and that which is lectotype of *P. subalutacea* suggests that the two may be sufficiently distinct to merit separate standing at the specific level, differing in color of fructification, in structure of context hyphae and of cystidia, and especially in spore dimensions.

PENIOPHORA SUBALUTACEA ssp. CINERACEA (Bourd. & Galz.) Bourdot & Galzin, Hym. de Fr. p. 293 ([1928])

P. glebulosa ssp. *cineracea* Bourd. & Galz., Bull. soc. mycol. France, **28**, 387 ([1913])

A "nov. subsp." in "slanted type" in the 1913 publication, this taxon was placed at the end of a list of several taxa apparently of the same rank, following *P. glebulosa* and immediately preceding *P. subalutacea*. A footnote to the description compares it, not with *P. glebulosa*, but with *P. subalutacea*. Saccardo (1925, p. 536) marked it "subsp.", adding "Pen. subalutaceae et Pen. glebulosae affinis". It was, it seems, to be considered a step between the two, and whether a subspecies of one or of the other did not matter, since the 1928 "Hyménomycètes" shows its name again in the "slanted type" of subspecific rank, but this time below *P. subalutacea*.

The 1913 citation gives the following data: "Aestate; ad Ericam arboream, Aveyron". Two authentic collections have been available in this study: from herbs. S and M.A.D. (*2734*), Bourd. *32017* ("Galzin *13427*, 9.VI.1913; ad ramos Callunae vulg., Balzaguet, Aveyron"); and from herb. M.A.D. (*2732*), Bourd. *8015* ("Galzin *6731*, 9.VII.1910, sur *Erica arborea*, Aveyron"). The latter collection seems to have been the only one Bourdot had at hand when he described the taxon. Dr. Donk has justifiably labelled his portion of it "part of type", and it is so designated here.

DESCRIPTION OF PART OF TYPE COLLECTION IN M.A.D. (*2732*)
(Bourd. *8015*, Galz. *6731*)

FRUCTIFICATION an interrupted reticulum, uniform and pale cream where young, strongly interrupted to the point of porosity and yellowing where older; browning in age especially where poroid appearance emphasized by dehydration causing withdrawal or disappearance of most of the hyphae from between 'walls' of 'pores' of mesh; separable; margin indeterminate. Condition rather poor; much of tissue collapsed, inhabited or overgrown by hyphomycete, but characterization possible. CONTEXT of loosely interwoven, distinct, mainly erect hyphae, nodose-septate, uniform in diameter, 2.8–4 μ wide; wall generally pronounced to somewhat thickened, colored by Congo red. CYSTIDIA emergent half-way or more; generally apical terminations of cystidiophores, sometimes with suggestion of basal furcation, usually false; more or less cylindrical, typically tapering sharply below, sometimes with trailing root; narrowing also above, but only slightly, to rounded apex; occasionally secondarily septate; length up to or exceeding 165 μ, width 8–11.5 μ at the widest part of stem (approximately midway), narrowing below to 4–5.5 μ at the basal septum, and above to 6.5–7.5 μ at apex; wall smooth, non-encrusted, thickened to as much as 3.5 μ at the broadest part of stem, narrowing gradually toward thin-walled apex, non-amyloid, staining strongly in Congo red, little affected by 10% KOH. BASIDIA mainly collapsed at maturity so that few reliable measurements available; apparently clavate, becoming more or less truncate at maturity, measuring ca. 20×6 μ; tetrasterigmate. BASIDIOSPORES few, cylindrical, straight to curved, 6.5–7.5 × 2–2.5 μ; wall thin, smooth, non-amyloid.

The affinity of this taxon to *P. subalutacea* is evident; but its maintenance at the subspecific level is questionable. When the only characteristics available are morphological, and two taxa appear to be distinguishable on the basis of these characters, it becomes necessary to specify how many or what kind of distinctions are sufficient for subspecific delimitation, and how many or what kind for designation at the specific level. What usually happens instead is that the taxonomist gives an instinctive evaluation of the differences, without explaining why these have greater or lesser significance than the differences between other taxa in the same genus. Although the instinctive decision is often right, unless it is backed by communicable evidence it is too authoritarian to be acceptable. It seems to me, therefore, that, in the absence of

contradicting evidence from cultures and mating tests (cf. Weresub and Gibson 1960), two distinguishable taxa should be regarded as different species.

The comparison between the lectotype of *P. subalutacea* and the type material of its "subspecies" *cineracea* shows differences between the two in choice of host, in gross appearance of their fructifications, in emergence of cystidia, and in furcation of their bases, and especially in the ratio of length to width of their basidiospores. If these differences are maintained when more material of both taxa is examined, it will be necessary to establish *cineracea* as a separate species.

PENIOPHORA GLEBULOSA ssp. CINERACEA var. MINOR Pilát, Bull. soc. mycol. France, **42**, 115 (1926) (n.v.)

Until material of this variety is re-examined for characters now used in distinguishing and relating the taxa of these groups, no useful attempt can be made to evaluate its status as a variety of *cineracea*. It seems to belong to the *P. subalutacea* group, as does the subspecies within which it has been placed, rather than to the "*P. glebulosa* group" in *Tubulicrinis*. But its classification at the varietal level may be questioned if the principle outlined above is adhered to, since the var. *minor* differs from *cineracea* at least in the possession of smaller, narrower cystidia, broader spores, and in host preference. However, no decision can be made here.

PENIOPHORA SUBALUTACEA ssp. LURIDA Bourdot & Galzin, Hym. de Fr. p. 294 ([1928])

The taxon is described as based on a specimen cited as "Autumne. Sur genévrier, Aveyron". The only collection available during this study is quite likely to have been the only specimen Bourdot had at hand: "Bourd. *9180ɛ*; Champs. de l'Aveyron; *12063* des recoltes de M. Galzin; Envoi du 13.XI.1912; sur genévrier; Vignoles". It appears justifiable to consider this collection the type specimen of this taxon.

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN PC AND M.A.D. (*2733*) (Bourd. *9180ɛ*, Galz. *12063*)

FRUCTIFICATION light pinkish cinnamon to pinkish cinnamon; soft-cushiony, dense, farinose-crumbly; separable. CONTEXT of coarse hyphae arranged in palisade, generally 4–5 μ wide with wall thickened as much as 0.7–1 μ, hyphal diameter sometimes swollen to as much as 9 μ, with wall up to 2 μ wide; large clamps at every septum. CYSTIDIA emergent to 60 μ; frequently with minute crystals and amorphous material clinging to apex, easily dislodged and apparently soluble in alkali; subconical in shape, broad at the base and furcate, or subfusoid, arising directly from a more or less erect cystidiophore; 40–80–100 × 4.5–8–12 μ broad just above the fork, 6–10 μ broad in the stem, and generally tapering upward to a somewhat cylindrical to conical apex 2.7–5.5 μ wide, thinner-walled; sometimes secondarily septate across lumen; wall more or less uniformly thickened, 0.7–1.3 μ in width, non-amyloid, staining throughout in Congo red, apparently unaffected by 10% KOH. BASIDIA produced in candelabrum arrangement, 20–24 × 4.7–6 μ, with four erect sterigmata. BASIDIOSPORES variable in shape: in face view, consistently ellipsoid, narrowing to the prominent apiculus; in profile or half-profile view, uniformly ellipsoid, or ovate, or oblong to ellipsoid with adaxially flattened side; 5–7 × 2.5–3.8 μ; some apparently engrained with or containing a resinous material; wall thin, smooth, non-amyloid.

Comparison between this specimen and that which is lectotype of *P. subalutacea* suggests that the two may be sufficiently distinct to merit separate standing at the specific level, differing in color of fructification, in structure of context hyphae and of cystidia, and especially in spore dimensions.

Xenasma Donk 1957

Following Rogers's proposal at a meeting of the Mycological Society of America in 1940, and Jackson's (1950*a*, 1950*b*) analysis of the members of section Athele of the genus *Corticium* and its close relatives, Donk (1957) erected the genus *Xenasma*, characterized most significantly by a waxy to gelatinous subiculum and the consistent presence of pleurobasidia (cf. Donk 1956*a*). At the same time (Donk 1957), he stated a preference for placing similar fungi but with a more or less arid subiculum in the genus *Epithele*.

Under the name *Xenasma*, we include the Tubuliferae which seem to possess pleurobasidia as a fundamental part of their development, whether or not there are "lyocystidia" present. Again, no definite conclusions regarding relationships are arrived at here.

Peniophora chordalis Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **115**(I), 1598 (1906)

Listed by Bourdot and Galzin as the first member of the Tubuliferae in their 1913 publication, but later ([1928]) transferred to their section Gloeocystidiales, this taxon has been treated recently by Jackson (1950*a*) under the name of *P. pruinosa* (Pat.) Jacks., and included by Donk (1957) in his new genus *Xenasma*. No further reference to it is necessary at this time.

PENIOPHORA CLEMATIDIS Bourdot & Galzin, Bull. soc. mycol. France, **28**, 383 ([1913]) (falso ut "clematitis")

At NY, there is a packet labelled *P. clematitis* and containing two notes: one stamped "G. Bresadola, Trento" and inscribed in what appears to be Bresadola's hand: "*Peniophora clematitis* B. & G. ex orig. ex.!"; the other, also apparently written by Bresadola: "*Clematis vitalba*, Aveyron, Galzin". Material from what appears to be the same collection is to be found as well in TRTC and D.P.R., also undated. Since the original description gives the host as "ad cortices emortuas Clematitis vitalbae frequens", this is apparently syntypic material and, though undated, was used (Weresub 1957) to establish the characters of the taxon.

Fortunately, however, two dated syntypes have appeared from herb. L: "Bourd. *7943*, sur *Clematis vitalba*, Trou d'Enfer, Galzin *9001*, 8 Mai, 1911" (Maire *123*, L *930.58-32*); and Bourd. *7946*, "*Clematis vitalba*, près St. Sernin, Galzin *9637*; 7, 1911" (L *930.192-48*). The former is here chosen as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN L (*930.58-32*)
(Bourd. *7943*, Galz. *9001*)

FRUCTIFICATION a barely noticeable white sprinkle, in more or less clearly delimited patches, tightly reticulate, dry, strongly hispid with needlelike cystidia, margin fading into a sparse scattering of cystidia. CONTEXT very sparse, of clamped hyphae as much as 3.5 μ in diameter, mainly intertwined with or invading substratal cells; occasional threadlike branches capped by a head of radiating acicular crystals, extending into the hymenial area. CYSTIDIA brittle, conical, as much as 150 μ long, 11–15 μ wide just above the truly multi-rooted base, emergent for the major part of their length, with or without climbing hyphae that are longer-celled and less frequently branched than the subicular hyphae; thickened wall distinctly lamellate: outer coat roughened, cracking, and splitting off, and eventually soluble in KOH, unstained by Congo red; the thin lamella inside the coat readily stained by Congo red, unaffected by 10% KOH; middle layer thick, clearing in KOH, eventually taking Congo red stain; inner layer thick or thin, pseudamyloid especially near the base where broadest, becoming amorphous in KOH; base of cystidium typically multi-rooted, with dense mat of clamped, frequently pseudamyloid hyphae closely associated; lumen broad or capillary. BASIDIA pleurobasidiate,

cylindric to flexuous subclavate, basally forked or several-rooted or appearing stalked, up to about 20 μ tall, as much as 7 μ broad at the widest, with four stout sterigmata up to 10 μ in length. BASIDIOSPORES broadly obovoid, subgloboid to globoid, 5–7.7 × 5–6.5 μ, delicately asperulate, aculei seen with difficulty and apparently soluble in KOH, or outer asperulate layer shed after long standing in alkali; non-amyloid. Occasional very large (14.8 μ diameter) basidiospores present, but no comparably large basidia seen.

A thorough discussion on this species is given by Boidin (1958*a*), where he considers the possibility that polyploidy explains the occurrence of the giant basidiospores and basidia in this taxon. These giant structures are the only characters on which it is possible to distinguish *P. clematidis* from *P. abietis* and *P. gladiola*. And yet, the description of *P. clematidis* states clearly that it is the small-spored taxon that Bourdot and Galzin had at hand and in mind. Therefore, whatever the fate of the large-spored taxon may be, it seems unavoidable to put into synonymy with *P. clematidis* both *P. abietis* and *P. gladiola* (cf. below).

Peniophora abietis (Bourd. & Galz.) Bourdot & Galzin, Hym. de Fr. p. 286 ([1928])

P. aegerita ssp. *abietis* Bourd. & Galz., Bull. soc. mycol. France, **28**, 383 ([1913])

The 1913 description of *P. abietis* begins its host-and-locale citation with "ad Abietem pectinatam, Arnac". An unquestionable syntype (Bourd. *7988*) is marked: "sur sapin pectiné, Aveyron, Arnac, 29.V.1911, Galzin *9468*". This number is here chosen lectotype.

DESCRIPTION OF PORTIONS OF LECTOTYPE COLLECTION IN S, TRTC, AND UPS (Bourd. *7988*, Galz. *9468*)

FRUCTIFICATION white, maculiform to confluent, thin, reticulate when young to tightly knit, closely adherent, strongly hispid with needlelike cystidia; margin indeterminate. CONTEXT very sparse; hyphae clamped, short-celled and twisted, ca. 2.5 μ broad; occasional short, threadlike branches, capped by a head of radiating acicular crystals, present. CYSTIDIA brittle, conical, 100–120 × 9–12 μ just above the truly multi-rooted base, emergent for the major part of their length, with or without climbing hyphae 1.5–2.5 μ in diameter; thickened wall distinctly lamellate: outer coat more or less roughened, sometimes easily flaked away or at least in part soluble in KOH, unstained by Congo red; the thin lamella inside the coat readily stained by Congo red, unaffected by 10% KOH; middle layer thick, clearing in KOH; inner layer thick or thin, more or less strongly pseudamyloid in Melzer's, becoming amorphous in KOH; base of cystidium typically multi-rooted, wall mainly composed of pseudamyloid material, so that concentration of pseudamyloidity is generally basal, though sometimes extending along the lumen almost to the apex of the cystidium, and occasionally penetrating all layers of the stem as well except the outer coat; lumen frequently broad toward base, more or less capillary in stem, occasionally occluded in apex. BASIDIA pleurobasidiate, cylindric to flexuous subclavate, basally forked or several-rooted; 13–18 × 5.5–6.3 μ, with generally four stout sterigmata ca. 6.3 μ long, arched at maturity. BASIDIOSPORES subgloboid to globoid, (4)5.5–7 × (4)5–6.3 μ; prominently apiculate; wall somewhat thickened, very delicately asperulate, aculei seen with difficulty, apparently soluble in KOH, non-amyloid.

It was Cunningham's (1955, p. 283) description of *P. gladiola* (cf. below) that drew attention to the crystal-capped hyphae ("paraphysate hyphae") which were abundant in the *P. gladiola* specimen examined by me, less so but certainly present in the lectotype collections of both *P. abietis* and *P. clematidis*.

Eriksson (1954) first described the pseudamyloidity of the cystidial walls in *P. abietis*. At the same time he referred to a similar reaction to Melzer's of the spore wall, to the bluing of the spore's inner wall and its aculei in cotton blue, and to the arrangement of the aculei in groups of 20. It must be admitted

that the keen eye of an Eriksson is needed to observe some of the fine details of structure in our fungi. I have been unable to confirm these observations except for a suggestion of pseudamyloidity in some of the spores of *P. gladiola*, and some staining by water-soluble aniline blue of the spore wall and aculei in this lectotype collection of *P. abietis*.

In the three taxa referred to here, cystidia, basidia, and spores are identical in structure, the size differences between the three type specimens bridged by overlaps. As far as I can see, there is so far no good evidence for maintaining them as distinct species.

Peniophora gladiola Cunningham, Trans. Roy. Soc. N.Z. **83**, 283 (1955) (TYPE: P.D.D. herb. *11484*) (n.v.)

DESCRIPTION OF PORTION OF PARATYPE IN TRTC
(on *Hedycarya arborea*, Auckland: L. Okataina; June 1951; J. M. Dingley; P.D.D. herb. *11444*)

FRUCTIFICATION white to pale buff, maculiform to confluent, thin, arid but tightly knit, closely adherent, strongly hispid with needlelike cystidia; margin rather abrupt. CONTEXT very sparse, of clamped hyphae, most of which are intertwined with or invading substratal cells, obscure; in some areas, hyphae branching profusely, frequently outlined in part or apically by a roughened layer, sometimes with additional engraining by acicular crystals; occasionally with threadlike branches capped by a head of radiating acicular crystals, sometimes extending into the hymenium. CYSTIDIA brittle, conical, 125–225 × 12–18 μ just above the rooting base, emergent for the major part of their length, with or without climbing hyphae that are longer-celled and less frequently branched than the context hyphae; thickened wall distinctly lamellate: outer coat more or less roughened, rather easily shelled off, the projections somewhat soluble in 10% KOH, unstained by Congo red; the thin lamella inside the coat readily stained by Congo red, unaffected by KOH; middle layer thick, clearing in KOH; inner layer thick or thin, more or less strongly pseudamyloid in Melzer's, becoming amorphous in KOH; base of cystidium typically multi-rooted, wall mainly composed of pseudamyloid material, so that concentration of pseudamyloidity is generally here, though sometimes extending along the lumen almost to the apex of the cystidium; lumen generally capillary throughout. BASIDIA pleurobasidiate, cylindric or flexuous subclavate, basally forked or several-rooted; 18–25 × 5–7 μ, with two to four stout sterigmata as much as 8 μ long, at maturity arched. BASIDIOSPORES typically globose to occasionally obovoid, (4.8)6–8.5 × (3.6)5.3–6.7 μ; prominently apiculate; wall occasionally showing slight suggestion of pseudamyloidity in inner layer, outer layer minutely asperulate, easily shelled away, projections more or less soluble in KOH, non-amyloid.

The description of a paratypic collection is included here in order to make possible a comparison between *P. gladiola*, *P. abietis*, and *P. clematidis*. There seems to be no doubt that we are dealing with one species, which must be called *P. clematidis*.

Cunningham's reference (*ibid.*, p. 285) to cystidia "enmeshed in a hyphal sheath as in *P. vermifera*" has not been confirmed in this study. The cystidia often act as climbing posts for hyphae, but the latter are no different from the context hyphae, except perhaps somewhat longer-celled, less frequently branched; whereas those in *P. vermifera* (q.v.) are of different nature from that of the hyphae of the context.

Peniophora hastata Cunningham, Trans. Roy. Soc. N.Z. **83**, 284 (1955) (not *P. hastata* Litsch., Oesterr. Botan. Z. **77**, 130 (1928)). [Since the former is a later homonym of the latter, the name of this taxon will be changed by Cunningham (*in litt.*, 1956).]

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(on *Griselinia lucida*, Auckland, N.Z.; May, 1951; J. M. Dingley; P.D.D. herb. *11442*)

FRUCTIFICATION white, maculiform to confluent, thin, tightly knit and closely adherent,

strongly hispid with needlelike cystidia, margin thinning but rather abrupt. CONTEXT sparse; subhymenial hyphae clamped, much-branched, short-celled, 2–3 μ in diameter [Cunningham: 3–3.5 μ], sometimes outlined in part or apically by a roughened layer. CYSTIDIA brittle, conical, 60–175 × 10–16 μ above the rooting base, emergent for the major part of their length, with or without climbing hyphae (1.3–2 μ in diameter), longer-celled and less frequently branched than the context hyphae; thickened wall distinctly lamellate: outer coat more or less roughened, unstained by Congo red; the thin lamella inside the coat stainable by Congo red, unaffected by KOH; middle layer thick, clearing in KOH; inner layer sometimes weakly pseudamyloid in Melzer's, becoming amorphous in KOH; base of cystidium typically multi-rooted; lumen generally capillary except in base. BASIDIA (few seen) cylindric to flexuous subclavate, bases obscured, 12–17 × 5–6 μ; sterigmata stout. BASIDIOSPORES of variable shape, from almost oblong to generally ellipsoid with adaxial side flattened, to obovoid or rarely subglobose; 5–7.5 × 3.8–5 μ; prominently apiculate; wall two-layered, outer layer usually clearly asperulate, less easily shelled away and projections less quickly soluble in KOH than in the case of *P. clematidis;* non-amyloid.

This species is known only from the type collection, a portion of which Dr. Cunningham was kind enough to deposit in TRTC. The material was found to be difficult to examine, a difficulty apparently shared by Cunningham who speaks of basidia and spores "soon collapsing". Comparison must be made with the descriptions of *P. clematidis*, *P. abietis*, and *P. gladiola*. The cystidia are like theirs, except that pseudamyloidity is very weak. Thread-like hyphae with heads of acicular crystals were not observed in *P. hastata*, but crystal-coated hyphae were. Observations on the basidia have been few, but what has been seen suggests a structure of the *P. clematidis* type and within its size range. Among the abundant spores are some of the typical subglobose *P. clematidis* shape, but most are more nearly obovoid, a form which may or may not be due to an incipient shrinking such as is carried to the point of obvious collapse in other spores, which then look somewhat ellipsoid with a flattened to concave side. The spore wall is of the same appearance as in *P. clematidis* spores, but the projections are more pronounced (perhaps because of shrinkage within the spore?) and less sensitive to alkali. Apropos of this is an observation made in a mount of *P. gladiola* material: a shrunken basidium carrying immature spores whose walls show much more pronounced projections than ordinarily occur in normal *P. gladiola* spores, and less sensitive to KOH dissolution.

It seems to me that the condition of the specimen of "*P. hastata* Cunn." could be ascribed to growth of *P. clematidis* under unfavorable circumstances of some kind. But until further collections clarify the role played by chance environmental hazards in producing the distinctions observed, it may be advisable to retain "*P. hastata* Cunn." as a taxon specifically separate from *P. clematidis*, though admittedly very close.

PENIOPHORA VERMIFERA Bourdot, Rev. sci. Bourbonnais, **23**, 13 (1910)

The original description of this taxon gives *Erica arborea* as type host, Aveyron as type locale. Bourd. *7962* ("Galzin *4200*; 3 Juillet, 1909"; Lloyd Collection *38022*—in BPI) fills the specifications for the type specimen, and is therefore designated as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN BPI
(Bourd. *7962*, Galz. *4200*)

FRUCTIFICATION in clearly defined patches of a creamy yellowing, tightly knit reticulum, dry; strongly hispid; somewhat separable. CONTEXT of some depth (ca. 30 μ) but difficult to separate into observable components; composed of subiculum of nodose-septate hyphae, uniform in diameter, ca. 2 μ, with firm walls, branching into subhymenial hyphae, thin-walled,

somewhat contorted and expanded in part to as much as 3.5 μ in diameter. CYSTIDIA stiff, needlelike cones, strongly emergent, 80–150 μ long, 11–18 μ in diameter just above the gradual to abrupt spread into a distinct true multi-rooting; narrowing uniformly to a more or less pointed apex; wall thickened, sometimes to the point of capillarity of the lumen throughout stem and roots but not in base; most of wall eventually shattered and soluble in 10% KOH; if left standing, thin outer layer finally colored by Congo red; negatively or positively amyloid, amyloidity usually only in circumluminar layer; covered or not by a deep sheath of fine hyphae (1–1.5 μ in diameter) with innumerable dendritic branches; more or less cylindrical, thin-walled structures, ca. 30×5.5 μ, present, some showing clamp at base, others more or less distinctly bi- to several-rooted, perhaps abnormal young cystidia, the normal showing wall-thickening at early stage. BASIDIA rarely clear throughout, but their pleurobasidiate structure distinct in some basidioles; broadly flexuous cylindrical to oblong-obovate, 20–30 × 7–10 μ, with four fleshy sterigmata, up to 10 μ long, strongly arched. BASIDIOSPORES flexuously subfalcate (banana-shaped), tapering apically from broader apiculate base, 20–24 × 3.5–4.2 μ; wall thin, smooth, non-amyloid.

Peniophora subglebulosa Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **116**(I), 823 (1907)

Following the description of *P. vermifera*, Bourdot and Galzin ([1928]) remark: "*P. subglebulosa* v. Hoehn. et Lit. . . . d'après la description et la figure, aurait des spores ovoïdes, 2–4 × 1.5–2 μ; mais l'habitat et les caractères externes sont identiques à ceux de *P. vermifera*. Nous avons étudié le spécimen original de *P. subglebulosa;* l'hyménium ne montrait ni spores, ni basides". In spite of this apparent acceptance of the *P. subglebulosa* specimen as a sterile *P. vermifera*, Bourdot and Galzin include *P. subglebulosa* in their key as a distinct species. The illustration of the latter (Hoehnel and Litschauer 1907, p. 824) depicts cystidia that might well have come from the fructification of *P. vermifera*, but spores that are quite different.

It appears that *P. subglebulosa* is a *nomen confusum*, and may be ignored in spite of publication prior to *P. vermifera*.

PENIOPHORA VERMIFERA var. PINICOLA Bourdot & Galzin, Hym. de Fr. p. 285 ([1928])

A portion of the type collection ("sur pin, l'Hospitalet; leg. A. Galzin *15517*, 2.VI.1914; Bourd. *32025*") is in M.A.D. (*10.103*). It is in poor condition and too scanty for thorough study. The cystidia seem to be typical of the species (conical, with thickened walls negative to lightly to definitely amyloid, and sheathed in characteristic dendritic hyphae) but no spores were observed.

It may be that enough differences exist for distinction at the varietal level between the form of the species growing on wood of heaths and that on wood of pine. But, to my mind, further evidence must be accumulated before any decision can be made.

PENIOPHORA DUSSII (Pat.) Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **116**(I), 749 (1907)

Hypochnus dussii Pat., Bull. soc. mycol. France, **15**, 202 (1899)

Epithele dussii (Pat.) Pat., Essai Taxon. p. 59 (1900)

This taxon was not included in the Tubuliferae by Bourdot and Galzin. Bourdot had never seen it, and merely mentioned it in passing in his discussion of *Epithele*. It is discussed here because of its obvious relationship to *P. vermifera*.

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN FH, NY
("sur un vieux stipe d'un *Alsophila aspera*, Bois des Bains, Tannes" [Guadeloupe]; [E. Duss 1899]. Pat. *3*)

FRUCTIFICATIONS more or less clearly delimited elliptic patches, white, crustose thin (or perhaps only apparently crustose because tightly adhesive to crustose epidermis of host), hispid with distinct, more or less uniformly spaced cystidia, cratered to substrate where individual cystidia have been lifted out or broken away. CONTEXT difficult to separate into components; where well-developed, of some depth (ca. 20 μ); composed of few subicular hyphae, nodose-septate, firm-walled, uniformly ca. 2 μ in diameter, leading to short-celled, much-branched, thin-walled hyphae, up to 2.8 μ in diameter; similar hyphae matted about base of cystidium and radiating upward, cytoplasm(?) pseudamyloid in some. CYSTIDIA stiff, needlelike cones, emergent almost total length, 55–115 μ long, 7–13.5 μ wide [Patouillard: 80×20 μ] just above the gradual to abrupt spread into truly bi- or multi-rooted base; narrowing uniformly to a blunt to pointed apex; wall thickened, but rarely to the point of capillarity of the lumen except in upper half of cystidium, eventually soluble in 10% KOH; encased in thick crystalline shell or plates, and covered with heavy weft of fine hyphae with innumerable dendritic branches; pseudamyloidity present in some cystidial bases, but apparently in lumen rather than wall, as well as in hyphae matted at the base. BASIDIA rarely clear throughout, but their pleurobasidiate structure almost certain; squat, flexuous, broadly cylindrical to obovate; ca. 15 × 6–10 μ; with four fleshy sterigmata, as much as 6 μ long, strongly arched. BASIDIOSPORES sublunate to flexuous subfalcate (banana-shaped), tapering apically from broader apiculate base, 7.8–15 × 2.5–3.5 μ [Patouillard: 6–7 × 2.5–3 μ]; wall thin, smooth, non-amyloid.

Peniophora vermicularis Wakefield, Kew Roy. Botan. Gardens Bull. No. **8**, 371 (1915)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN K
(on fern petiole; Whakarewarewa, N.Z.; 1914; W. N. Cheesman)

FRUCTIFICATIONS in clearly defined patches, white, very thin and fragile, hispid with distinct, more or less uniformly spaced cystidia; closely adherent to substrate. CONTEXT inconspicuous, difficult to separate into observable components; subicular hyphae nodose-septate, firm-walled, uniform in diameter to 2.5 μ, leading to subhymenial hyphae, thin-walled, contorted. CYSTIDIA stiff, needlelike cones, emergent almost total length, 50–75 μ long, 10–11 μ wide [Wakefield: 45–70 × 8–14 μ] just above the gradual to abrupt spread into a rounded-off or clearly truly bi- or multi-rooted base; narrowing uniformly to a blunt or pointed apex; wall thickened, typically to the point of capillarity of the lumen throughout stem and roots but not in basal spread, eventually soluble in 10% KOH; encased in thick crystalline shell or chips, and covered with heavy weft of fine hyphae with innumerable dendritic branches; pseudamyloidity of extreme base observed in one cystidium. BASIDIA rarely clear throughout, but their pleurobasidiate structure almost certain; flexuous cylindrical, 12–17 × 5–6 μ; with four fleshy sterigmata, strongly arched. BASIDIOSPORES sublunate to flexuous subfalcate (banana-shaped), tapering apically from broader apiculate base; 7.5–10 × 2.5–3 μ [Wakefield: 9–11 × 2–3 μ]; wall thin, smooth, non-amyloid.

It was Professor Rogers who pointed out to me that this taxon was possibly identical with *P. dussii*. A comparison of the two type collections has established this synonymy, in spite of the differences apparent in the descriptions; i.e., the generally greater size of all structures in *P. dussii*, but with an overlapping of the size ranges in *P. vermicularis*, and the more consistent occurrence of pseudamyloidity in *P. dussii*. But being able to accept these as the normal variation possible within a species, I do not hesitate to discard the name *P. vermicularis* as a synonym of *P. dussii*.

EPITHELE HORRIDULUM Rick, Broteria Ciencias Naturalis, **9**(36), 148 (1940) (n.v.)

Referring to Hoehnel and Litschauer's (1907, p. 750) erroneous description of *P. dussii*, Rick states that the taxon he describes under the name of *E. horridulum* may be identical with that species. However, if his description of the spores is accurate ("trianguloso-irregularibus 5×4 μ grosse apiculatis"),

there is no possibility that the two are synonymous.

Species of Other Affinities

PENIOPHORA FARINOSA (Bres.) Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **117**(I), 1095 (1908)

Kneiffia farinosa Bresadola, Ann. Mycol. **1**, 105 (1903)

Burt ([1926]) designated as "part of type" a collection (now at FH) labelled apparently by Bresadola as follows: "*Peniophora farinosa* Bres. n.sp.; ad ramos Ulmi; Polonia rossica; Oct. 1901, Coll. Eichler", with the following added in Burt's hand: "Type: *Kneiffia farinosa* Bres.", the "*P. farinosa*" marked by Burt as a 'herbarium name'. Burt's published statement (*loc. cit.*) fixes the lectotype of this species, since there is no clear-cut reason, so far, to discard his choice.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN FH
(Eichler, Oct. 1901)

FRUCTIFICATION cream to yellowish, with white margin; soft, farinose, distinctly hispid with long-emergent cystidia; a separable, uneven reticulum with open meshes marginally, but dense in age, losing almost all signs of reticulation. CONTEXT loose, of thin-walled, easily collapsed hyphae, varying in diameter from 3 to 6 μ, with prominent clamps. CYSTIDIA emergent to 70 μ above the surface of the hymenium, as viewed under the dissecting microscope; brittle, more or less cylindrical to gradually tapering upward but with apex obtuse; as much as 120 μ long, base of hyphal diameter or expanded to a width of 9–11 μ, not furcate, apex 6–7 μ wide; wall thickened throughout length and over apex, non-amyloid, apparently at least three-layered: an external shell of roughened crystalline material, disappearing after standing in alkali solution, a more or less thickened layer inside it, staining in Congo red and rather insensitive to alkali, and a more or less thickened internal layer which apparently swells and eventually disintegrates in KOH; lumen capillary or broad. BASIDIA mainly collapsed, flexuous elongate, as much as 8 μ wide, with four fleshy arcuate sterigmata. BASIDIOSPORES subglobose to broadly ellipsoid, 6–8.5 $\times$ 4.8–8 μ (one 11$\times$9 μ seen); prominent apiculus sometimes somewhat askew; cytoplasm granular; wall smooth, thickened, non-amyloid, staining in Congo red.

Accompanying *Aegerita candida* Pers. ex Fr.: yellow bulbils standing as tall as 200 μ, but subgloboid or ovoid portion ca. 100–150 μ in diameter; in clumps. Each bulbil composed of centrifugally radiating, branching chains of progressively larger cells; prominently clamped; the apical cell in each chain swollen, subglobose, and stalked or broadly clavate; these cells closely compacted, their walls thin or more or less thickened, stained by Congo red, their globes measuring as much as 18$\times$11.5 μ; cytoplasm pseudamyloid.

On the basis of this collection, Burt reported this species as synonymous with *P. candida*. His choice of lectotype and his synonymy have not been accepted, primarily because the original description of *P. farinosa* did not refer to an accompanying *Aegerita* state.

But in this study, three other authentic collections of *P. farinosa* have been examined, in addition to the lectotype. Bres. *12* (in S) is attended by bulbils of *Aegerita tortuosa* Bourd. & Galz. Bres. *12-a* (also in S) is closely associated with bulbils of *A. candida*. As for an unnumbered Eichler collection from the Bresadola herbarium in UPS, I have no notation of the presence of bulbils; they may be lacking.

In spite of the fact that Bresadola himself did not distinguish between his collections on the basis of presence or absence of an *Aegerita* stage, it has been the practice to identify, as *P. candida*, specimens associated with *A. candida*, and, as *P. farinosa*, those lacking this imperfect state.

In support of this treatment, we have Lacy's (1937) studies. His cultures from such a collection of "*P. farinosa*" (TRTC *10844*) did not mate with his cultures from a collection of "*P. candida*" (TRTC *9918*); nor did the former

produce the *Aegerita* state in culture, whereas the latter did. And yet, what appears to be a similar collection (Fungi Exs. Suec. *2133*), cultured by Eriksson (cf. Lundell and Nannfeldt 1953), when crossed with typical "*P. candida*", produced, in all pairings, dicaryotic mycelium with clamps.

So far, only Lacy's work denies Burt's synonymy. Hoehnel and Litschauer's distinction (1908), that of a difference in size of spores, seems to be a matter of overlapping range. Until further studies confirm Lacy's observation of the presence of an intersterile but morphologically indistinguishable group, I feel we are justified in taking Eriksson's mating studies as having established the identity of the two taxa. Therefore, Burt's choice of lectotype is accepted here; and the species must take the earlier valid name of *P. farinosa*.

Peniophora candida Lyman, Proc. Boston Soc. Nat. Hist. **33**, 168 (1907)

In February 1907, Lyman described his cultural and developmental studies of a basidiomycete found in nature associated with an imperfect stage which Persoon had called *Aegerita candida*. Lyman "transferred" the imperfect epithet to the perfect genus; but since such a transfer is, to my mind, invalid under Art. 59 of the code (cf. Deighton 1960; but also Donk 1960), the name is rated as that of a new species published by Lyman in 1907, rather than a transfer from the imperfect name validated by Fries in 1821.

The Lyman collections sent by him to Burt were certainly his syntypic material (cf. Lyman, *ibid.*, p. 167: "specimens . . . were sent to Prof. Burt, who pronounced it a new species of *Peniophora*"). Lyman's statement, "Thus far the perfect condition has been reported only from Cambridge, Mass. and Hanover, N.H.", makes it necessary to designate as lectotype his Hanover collection in the Burt herb., because the other Lyman specimens there were collected, not at Cambridge, but in Waverly, Mass. It may have been a slip of the pen by Lyman, but to avoid unnecessary questioning, I have selected the Hanover specimen as lectotype.

DESCRIPTION OF PORTION OF LECTOTYPE COLLECTION IN FH
(on *Alnus*, Hanover, N.H., Oct. 1904; G. R. Lyman)

FRUCTIFICATION pale cream, dense and smooth where mature, reticulate in younger portions, marginally only an indefinite farinosity. CONTEXT of closely branched, distinctly palisaded hyphae, up to 5.5 μ in diameter, with walls thin to firm, stained by Congo red; clamps prominent; tissue easily squashed, exuding cytoplasm. CYSTIDIA very rare [Burt: 60–70 × 10–14 μ], only a few segments observed, conical, as much as 65 μ long, 15 μ wide at the base (where seen); wall heavily thickened, outer layer thin, crystal-engrained, and rough; lumen capillary. BASIDIA distinct, flexuous cylindrical to subclavate, up to 30 μ long, as much as 7.2 μ wide, with four fleshy sterigmata, erect, slightly arched, up to 6 μ long. BASIDIOSPORES subglobose, 5–8.8 × 5–7.3 μ [Burt: 6–7 × 5–6 μ]; prominent apiculus somewhat askew; cytoplasm granular; wall smooth, thickened, non-amyloid, staining in Congo red.

Accompanying *Aegerita candida* Pers. ex Fr.: yellow bulbils up to ca. 150 μ diameter, subglobose or ovoid, appearing sessile on substrate, but attached at a single basal point, sprinkled individually or in groups, mainly around or at the observable margin of the perfect fructification. Each bulbil composed of centrifugally radiating, branching chains of progressively larger cells; prominently clamped; the apical cells in each chain swollen, subglobose, stalked or not; these cells closely compacted, their walls thin or more or less thickened, stained by Congo red, their globes reaching a diameter of as much as 15 μ; cytoplasm pseudamyloid.

A comparison of this description with that of the lectotype collection of *P. farinosa* leaves no doubt of the synonymy of the two taxa so typified. With the acceptance of the typification presented here, the synonymy, and the interpretation of Art. 59 of the International Code given above, the name

P. candida must give way to the prior claim of *farinosa* as the legitimate epithet for this species.

Peniophora aegerita Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **116**(I), 810 (1907)

In May 1907, Hoehnel and Litschauer discussed the perfect stage associated with *Sclerotium aegerita* Hoffm., and made the "transfer" to the genus *Peniophora*, just three months after Lyman's publication of *P. candida*.

Although I have not seen what may be considered syntypic material of *P. aegerita*, the authentic specimens which have been available to me, determined by Litschauer, certify the accuracy of the decisions of Bourdot and Galzin ([1928]) and of Burt ([1926]) that *P. candida* and *P. aegerita* are one. *P. aegerita* is therefore a later synonym of *P. candida*, and both are discarded here as synonyms of *P. farinosa*.

Peniophora odorata sensu Burt, Ann. Missouri Botan. Garden, **12**, 289 ([1926])

Stereum odoratum sensu Karsten, Bidr. Känned. Finl. Nat. Folk, **25**, 309 (1876) (not *S. odoratum* (Fr.) Fr., Epicr. p. 553 (1838)—teste Bresadola, Hym. Kmet, p. 108 (1897); Hoehnel and Litschauer, Sitzber. **116**(I), 765 (1907); Burt, *loc. cit.;* Rogers and Jackson, Farlowia, **1**, 320 (1943). Nor *S. karstenii* Bresadola, I.R. Accad. Agiati Atti, **111**(3), 108 (1897)—teste Burt, *loc. cit.*, pp. 286 and 289; Rogers and Jackson, *loc. cit.*)

Xerocarpus odoratus sensu Karsten, Rev. mycol. **3**(9), 22 (1881)

Phanerochaete odorata sensu Karsten, Bidr. Känned. Finl. Nat. Folk, **48**, 427 (1889)

Although the Karsten collection designated by Burt as type of this taxon (at FH, "in ligno pini, Mustiala, Karsten") has been available to me, it seems superfluous to describe it here, since the name itself has been interred (cf. Rogers and Jackson 1943) on account of its misapplications through its long history outlined in the synonymy list above. A new type with a new name, *P. luna*, has replaced *P. odorata*.

PENIOPHORA LUNA Romell ex Rogers and Jackson, Farlowia, **1**, 320 (1943)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN FH

(on rotten wood of pine; Lappland, Sweden; Aug. 17, 1910; Romell *413*)

FRUCTIFICATION in clearly delimited patches; cream to pale salmon-cream; cushiony fibrous; fissured to substrate; surface velvety to furry; more or less separable; margin furry, radiating, depressed, determinate. CONTEXT 700 μ deep, compact, apparently not zonate; composed of closely interwoven but palisaded hyphae and cystidia; hyphae ca. 3.5 μ in diameter, with prominent, sometimes "ansiform" clamps, some with regularly spaced secondary simple septa; walls emphasized to broadly thickened; non-amyloid, staining in Congo red. CYSTIDIA embedded or emergent, clavate or somewhat broader in stem than at apex, as much as 200 μ in length, of hyphal diameter at basal clamp, broadening to a maximum width of 7–11(14) μ; more or less uniform or with regular strangulations; secondary simple septation across lumen in some cystidia; wall moderately to broadly thickened, sometimes as much as 3.5 μ, usually thinning toward apex; non-amyloid, staining in Congo red, little affected by 10% KOH. BASIDIA abundant, long clavate, up to as much as 100 μ long or more, 8–9 μ wide, with four fleshy sterigmata up to 8.2 μ long. BASIDIOSPORES generally not deeply stained by phloxine, apparently many not completely filled with cytoplasm and so perhaps not quite normal in shape; obovoid when immature and still attached; when mature and free, mainly askew-ellipsoid or narrowly half-moon shaped; 14–15.2 × 4–5.5 μ [Romell: 18–20 × 5–6 μ; Burt: 13–15 × 5–6 μ]; wall thin, smooth, non-amyloid.

PENIOPHORA INCRASSATA Malençon, Bull. soc. mycol. France, **68**, 316 (1952)

DESCRIPTION OF PORTION OF TYPE COLLECTION IN NY
(on wood of decomposing *Cedrus;* Bon Jihrirt, Moyen-Atlas, Fr. Morocco; 15.XI.1944; Malençon *2021*)

FRUCTIFICATION rose-cream, cushiony soft-fibrous; cracked; surface velvety to furry; separable; margin determinate. CONTEXT thick (1 mm and more in depth), dense, zonate, with large (up to 30 μ long) several-faceted, roughly diamond-shaped crystals scattered throughout, sometimes massed around the base of a zone; tissue mainly collapsed and difficult to revive, somewhat yellowish, composed of closely interwoven but palisaded hyphae and cystidia; hyphae ca. 3.5 μ in diameter, with prominent, sometimes "ansiform" clamps; walls emphasized to broadly thickened, sometimes to the point of capillarity or even occlusion of the lumen; non-amyloid, staining in Congo red. CYSTIDIA embedded or emergent, more or less blunt-clavate though sometimes broader in stem than at apex; as much as 300 μ in length, but more frequently shorter, of hyphal diameter at the basal clamp, broadening only gradually to a maximum width of 7–10 μ; more or less uniform or with regularly spaced strangulations; secondary simple septation across lumen frequent; wall moderately to broadly thickened, sometimes as much as 3.5 μ, uniformly broad or thinning toward apex, outer layer sometimes delicately roughened, becoming smooth in all mounting media; non-amyloid, staining in Congo red, not immediately or drastically affected by 10% KOH. BASIDIA mainly unrecognizably collapsed and emptied, long clavate, as much as 70 μ long and at least 7.5 μ broad, tetrasterigmate. BASIDIOSPORES mainly only lightly stained by phloxine, probably not completely filled with cytoplasm and therefore deformed in outline, apparently normally subcylindrical to reniform but tending to collapse into a slightly curved elliptical or even subfusiform shape; 10–12.5(14) × 3–4 μ; wall thin, smooth, non-amyloid.

It is unfortunate that little reliance can be placed on the above description of the spore shape and size in this taxon, for there appears to be distortion of the former and hence probably also of the latter. The description of the spores found in the examined portion of the type material of *P. luna* may be suspect for the same reason: the phloxine is not taken up uniformly in most of the spores seen, indicating the likelihood of a distorted outline. This makes comparison of the taxa difficult, since, as Malençon (1952) himself points out, the only possible distinction is on the basis of shape of spores.

Examination of the three paratypes of *P. incrassata* (Malençon *243*, *1240*, and *2292*—portions in NY) extends the spore width to 5 μ and confirms the subcylindrical to reniform shape as the normal shape of the spores of that taxon. Examination of the paratypes of *P. luna* (Romell *412* and *369*—in FH) extends its spore dimensions to 17.5×6 μ and reaffirms the observed variations in spore shape, from the occasional *incrassata* outline to a half-moon or orange-section to a subfusiform or fusiform-elliptic shape. These are details to be kept in mind when the specific limits of these taxa come under consideration.

Meanwhile we have two type specimens which are distinguishable, though apparently only on the basis of some slight difference in spore shape and a difference in range of spore magnitude. For the present, therefore, *P. incrassata* and *P. luna* may be maintained as separate species, until a summation is made of the morphologic evidence provided by the examination of as much material as can be made available of both taxa. Eventually, it is to be hoped that information from interfertility studies will help us make our final decision.

PENIOPHORA LONGICYSTIDIA Litschauer, Oesterr. Botan. Z. **77**, 131 (1928)

DESCRIPTION OF PORTIONS OF TYPE COLLECTION IN UPS, NY
(on rotted wood of conifer; Troppberg near Purkersdorf, Austria; 12.VIII.1925; K. Keissler and V. Litschauer; Litsch. *223*)

FRUCTIFICATION creamy where young, hispid, and densely reticulate, becoming reddish to deep brown in age, velvety to smooth and membranous to ceraceous to almost corneous;

margin more or less fibrillose. CONTEXT composed of a shallow, indistinctly horizontal subiculum with a deep trama (as much as 300 μ and more) of compactly palisaded hyphae and cystidial stems, when mounted in KOH eventually looking as if immersed in a matrix of gelatinized hyphal walls; hyphae 3.5–5.5 μ in diameter, prominently nodose-septate, tortuously branched and intertwined, with irregular swellings; wall pronounced or thickened, measuring as much as 2 μ when swollen in alkali, eventually partly stained by Congo red, questionably amyloid in part. CYSTIDIA clavate or broadly subfusiform but still obtuse at the apex, sometimes irregularly strangulated; as much as 200 μ long and more, 7–11(13.5) μ broad at the widest point, originating deep in the trama as the modified apical end of a cystidiophore, in young hymenium emergent as much as 100 μ, with scattered crystals encrusting exposed portion, but largely embedded in older parts of fructification; frequently secondarily simple septate; wall thickened, sometimes to the point of capillarity of lumen in lower segment, usually thinning upward to apex, in Melzer's partly yellowed, partly questionably amyloid; inner layers of wall sensitive to KOH but not completely dissolved, shell left standing and eventually colored by Congo red. CYSTIDIOLES more or less sphaero-pedunculate to obovate, as much as 50 μ tall though usually shorter, swollen heads (up to ca. 10 μ wide) on generally narrowed hyphal stalks, with or without secondary septation, frequently emptied wholly or in part, the cytoplasm often heterogeneously coagulated and highly refractive; wall firm to thickened, apparently somewhat amyloid especially apically, also staining in Congo red. BASIDIA (few seen in condition for measurement) clavate, 30–40 × 5.5–7 μ. BASIDIOSPORES broadly ellipsoid to subgloboid, with or without an adaxial flattening and slight incurving; (5)5.8–7.5(8) × 3–4.2(4.5) μ; cytoplasm frequently heterogeneously coagulated; wall thin, smooth, non-amyloid.

PENIOPHORA VIRIDIS (Preuss) Bres., *in* Bourdot and Galzin, Hym. de Fr. p. 295 ([1928])

Thelephora viridis Preuss, Linnaea, **24**, 152 (1851) (teste D.P.R., nec Berk. *in* Hook., Fl. Tasm. **2**, 258 (1860))

Corticium viride (Preuss) Fr., Hym. Eur. p. 652 (1874) (teste D.P.R., nec Berk., *in* Hook., Fl. Nov.-Zel. **2**, 184 (1854); nec (Link) Bres. ex Hoehn., Oesterr. Botan. Z. **54**, 428 (1904) (≡*Chloridium viride* Link, Handb. Gawächse, **3**, 465 (1833)); nec Bres., *in* Hoehnel and Litschauer, Sitzber. **116**, 836 (1907))

No Preuss material of this taxon has been available in this study. The present concept arises from examination of specimens described by Bourdot and Galzin (*loc. cit.*). Bourd. *8686*, in PC, marked as communicated by Bresadola, 24.III.1912, is labelled "*P. viridis* (Preuss) Bres. ... mea sensu, orig. non vidi; ad ligna acifolia, Suecia, Romell", with an almost complete description that coincides with that published in 1928. Another specimen from PC (Bourd. *40553*¢) turns out to be that which Bourdot and Galzin describe in their footnote to the specific description (A. de Crozals, 15.IV.1926). Since no type can be designated from this material, no description is given here.

PENIOPHORA LIVIDA Burt, Ann. Missouri Botan. Garden, **12**, 239 ([1926])

At TRTC, there is a portion of an Elias Fries collection sent by Dr. Seth Lundell to Dr. H. S. Jackson, with an accompanying note: "Fragment ex Herb. Elias Fries. One of the two specimens named '*Corticium lividum* Pers.', collected by Fries in Femsjö, and with Fries' own handwriting. This is what Burt later named *Peniophora livida* (Fr.) Burt. But '(Fr.)' must be dropped and the sp. named only *P. livida* Burt". The other collection referred to by Lundell is the true *Corticium lividum*.

DESCRIPTION OF PORTION OF TYPE COLLECTION IN TRTC
(Femsjö, Sweden, Elias Fries)

FRUCTIFICATION glaucous pinkish-tan, a smooth, vernicose film, somewhat separable. CONTEXT ca. 100 μ deep, composed of a shallow horizontal subiculum plus a relatively deep,

erect trama; hyphae up to 3 μ in diameter, tortuous and branched, mainly emptied of cytoplasm and so uncolored by phloxine, others with narrow, threadlike, phloxine-stained lumina and more or less pronounced or thickened walls; these in part eventually stained lightly by Congo red, but agglutinated apparently through gelatinization of part of the wall and therefore indistinct, further gelatinized when mounted in KOH. CYSTIDIA rare, subfusoid, as much as 55 μ tall and 6–8 μ wide at the broadest point about midway or lower, tapering upward to a more or less pointed apex, with or without a minute apical bulb, emergent portion engrained or not with fine crystals; basally with a trailing root, occasionally bifurcate but showing no sign of true bi-rooting; wall non-amyloid, thickened, probably normally no more than 1–1.5 μ at widest, but swelling in alkali to as much as 3 μ, staining in Congo red. BASIDIA (few observed) more or less clavate, tetrasterigmate, with firm walls, ca. 25×3 μ. BASIDIOSPORES (very few seen) cylindrical, slightly curved, 6×2.5 μ; wall thin, smooth, non-amyloid.

Peniophora livida ssp. *lactinea* Bourdot & Galzin, Hym. de Fr. p. 284 ([1928])

No authentic material of this taxon has been available in this study. The description suggests that whatever material came to hand looking like it would probably be identified as *P. livida*, since there appears to be no constantly correlated clear distinction between the white and the more highly colored forms of this species, at least not sufficient for subspecific differentiation.

Peniophora segregata Bourdot & Galzin, Hym. de Fr. p. 284 ([1928])

The one citation in the description of this species is: "Septembre; sur sapin, environs de Turini, Alpes Mar. (E. Gilbert)". PC provided, on loan, a collection marked: "Bourd. *39497*¢; Champs. des Alpes maritimes; *P. segregata* n.sp.; sur bois de sapin; Turini; legit. E. Gilbert *1204;* Commtde. IX. 1925". This may certainly be taken as type and only specimen of *P. segregata*.

According to the description, this taxon differs from *P. livida* by the possession of spores characterized as oboval, often briefly narrowed to the base, 4.5–5 × 4 μ. Examination of the type collection yields no spores of this kind; the only ones present which appear to belong are cylindrical, slightly curved, 6×2 μ, which is typical for *P. livida*. On the basis of gross appearance of the fructification, its texture and structure, and the form of cystidia, basidia, and now spores as well, this is a collection of *P. livida*.

The name *P. segregata* Bourd. & Galz. may therefore be considered a ***nomen confusum***, since the description is apparently based on the fructification of one organism and the spores of another; or it may simply be put into synonymy as an incorrectly described *P. livida*. The latter policy appears desirable in this instance, since it provides a Bourdot and Galzin epithet for the species in the event that it be transferred to *Phlebia* (cf. Donk 1957, *et al.*) where the epithet *livida* is preoccupied.

PENIOPHORA POLONENSIS (Bres.) Hoehnel & Litschauer, Ann. Mycol. **4**, 292 (1906)

Kneiffia polonensis Bresadola, Ann. Mycol. **1**, 103 (1903)

This taxon was included in the Tubuliferae in the 1913 publication of Bourdot and Galzin, and removed to the Ceraceae in 1928. A somewhat hypochnoid fructification with hyphal, nodose-septate cystidia, relatively large basidia, and broad spores, this fungus has been transferred by Boidin (1957) to *Pellicularia* and by Donk (1957) to *Hyphoderma*. Wherever it belongs, it seems to bear little relationship to any of the species of the Tubuliferae, and is therefore omitted from consideration here.

Peniophora abietis var. *pinastri* Bourdot & Maire, Bull. soc. mycol. France, **36**, 73 (1920)

No authentic material of this taxon has been available during this study. But the description suggests that Bourdot and Galzin's later ([1928]) treatment of it as subspecific to *P. orphanella* (included by Donk (1957) in *Hyphoderma*) places it among closer relatives than "*P. abietis*".

Peniophora peckii Burt, Ann. Missouri Botan. Garden, **12**, 291 ([1926])

Considered by Burt a member of the "*glebulosa* group", this taxon, with its rough, thick-walled spores, has been shown by Rogers and Jackson (1943) to be the quite unrelated *Corticium punctulatum* Cke, which is included by Eriksson (1958) in *Hypochnicium*.

PENIOPHORA SUBSULPHUREA (Karst.) Hoehnel & Litschauer, Sitzber. Akad. Wiss. Wien, **115**(I), 1561 (1906)

Corticium subsulphureum Karsten, Soc. Fauna & Flora Fenn. Medd. **6**, 12 (1881)

Xerocarpus subsulphureus (Karst.) Karst., Bidr. Känned. Finl. Nat. Folk, **37**, 138 (1882)

Kneiffia subsulphureus (Karst.) Bresadola, Ann. Mycol. **1**, 105 (1903)

Bourdot and Galzin included this taxon in the Tubuliferae of their 1913 publication, but excluded it in 1928, transferring it to the Membranaceae. Collections by Karsten ("Mustiala, in ligno pini"), Galzin ("II.1908; Bres. *5396*"), and Litschauer (*39*) were examined, the first two in NY, the latter in D.P.R. and TRTC. All are of a fungus with hyphal cystidia and amyloid spores, characteristics which remove it from among the Tubuliferae, heterogeneous as the section may be. Pouzar (1959) has made *P. subsulphurea* the type species of his genus *Amylocorticium*.

PENIOPHORA PILOSA Burt, Ann. Missouri Botan. Garden, **12**, 291 ([1926])

DESCRIPTION OF PORTION OF TYPE COLLECTION IN FH
(on coniferous wood; Ithaca, N.Y.; 1902; G. F. Atkinson *14415*)

FRUCTIFICATION a creamy white reticulum, alternately closely knit and aereolate, somewhat farinose, sparsely villose in profile. CONTEXT thin, ca. 50 μ deep at most, composed of rather loosely interlaced, nodose-septate hyphae, 2.5–3.5 μ in diameter, thin-walled. CYSTIDIA usually emergent as much as half their height, more or less cylindrical, up to 85 μ in length, 4.5–9 μ in diameter (rarely, emptied, measuring as much as 14 μ) [Burt: 60–100 $\times$ 4.5–7 μ], narrowing below more or less abruptly to a short stem of hyphal diameter, rounded at apex, frequently with strangulations producing a single apical bulb, a pair or short series of bulbs 7–15 $\times$ 3.5–5.5 μ; wall uniformly thin to firm, non-amyloid, staining lightly in Congo red. BASIDIA somewhat flexuous cylindrical, slightly expanded above (one-third from base), ca. 35 $\times$ 5.5–7 μ, frequently on a short stem of hyphal diameter; with four fleshy, erect sterigmata. BASIDIOSPORES oblong-ellipsoid; 7–9 $\times$ 3.5–5 μ; wall smooth, pronouncedly thickened, apparently hyaline, clearly pseudamyloid; apiculus pronounced.

Burt listed *P. pilosa* with the "*glebulosa* group". But none of its characters point to such a relationship. The so-called cystidia look like the structures that have been called gloeocystidia in *Corticium litschaueri*, *C. hydnans*, and *Radulum orbiculare*, but are more markedly emergent. The basidia develop more or less as Lentz (1957) described for basidia of *Coniophora*. The spores also approach those of *Coniophora* in structure, but they appear to be colorless and are distinctly pseudamyloid.

Reference to pseudamyloidity in the colorless spores of resupinates is rare.

As mentioned earlier, Eriksson (1954) reported pseudamyloidity of the inner wall of spores of *P. abietis*, and I have observed faint evidence of it in spores of *P. gladiola*. Kotlaba and Pouzar (1959) record spore pseudamyloidity in *Poria medullaris*. And it is also reported by Pouzar (1958) and has been observed in our laboratory in the spores of *Merulius molluscus* Fr., type of his new genus *Leucogyrophana*, which he places in the family Coniophoraceae. It may be here or hereabouts that *Peniophora pilosa* will find its place, although its texture is altogether different from that of *M. molluscus*.

It must be noted that the collection from East Galway listed by Burt as a paratype of *P. pilosa* does not belong to that species.

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SOMATIC MITOSIS IN A BASIDIOMYCETE[1]

E. W. B. Ward and Kathleen W. Ciurysek

Abstract

The nuclei in stained preparations of the mycelium of an unidentified basidiomycete were shown to divide by mitosis. Typical stages of division with the exception of early prophase were clearly distinguished and the haploid chromosome number was shown to be 4. The view that the fungus is a basidiomycete was confirmed by the demonstration of conjugate nuclei and clamp connections.

Introduction

Nuclear division by meiosis and mitosis has been demonstrated in the process of sexual reproduction in many fungi. Olive (12), on the basis of a survey of the literature, concluded that the nuclei in the vegetative mycelium of fungi also divide by mitosis. However, in several recent publications (1–8, 14, 15, 19) it has been suggested that somatic nuclear division in fungi does not follow the normal mitotic sequence.

In this paper an account is given of mitotic division in the hyphae of an unidentified basidiomycete. This fungus, a causal agent of snow mold, has never been observed to fructify, but the cytological observations described here confirm the view (21) that the fungus is a basidiomycete.

Materials and Methods

The culture of the unidentified basidiomycete used here was from the W_1 isolate described by Ward *et al.* (21). For the purpose of staining, it was grown on a medium composed of malt extract (Difco), 5 g; yeast extract (Difco), 5 g; *d*-glucose, 15 g; and distilled water, 1 liter, in either still or shake culture at 15° C as described by Ward and Colotelo (20). Synthetic media gave less-satisfactory results, as the mycelium was finer and the nuclei were more difficult to observe. Optimum results were obtained using shake cultures incubated for 5 to 8 days, a period during which growth was particularly rapid and nuclear divisions especially numerous. Still cultures were incubated for 8 to 14 days.

After incubation cultures were centrifuged to remove the culture medium, suspended in distilled water, and homogenized (20). The resulting homogenate consisted of masses of short hyphal segments each containing several entire cells, the viability of which has been demonstrated elsewhere (20). Films of homogenate were spread on the surface of the microscope slides, air-dried, and fixed for 10 minutes in a mixture of absolute alcohol, glacial acetic acid, lactic acid 6:1:1 (17). The preparations were then washed in 95% ethyl alcohol followed by 70% ethyl alcohol.

Staining was carried out by the HCl–Giemsa method, in general following the procedure described by Hrushovetz (9). Certain modifications of this

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procedure were required for optimum results with the organism studied here. After removal from 70% alcohol the preparations were washed in distilled water, placed in *N* HCl at room temperature for 5 minutes, hydrolyzed in *N* HCl at 60–61° C for 6 minutes, washed in five changes of distilled water, suspended in a phosphate buffer (pH 7.2) for 5 minutes, and stained in a solution of Giemsa's stain for approximately 25 minutes. The slides were washed thoroughly with distilled water and then with buffer, drained, flooded with Abopon, and covered with a cover slip.

The buffer was prepared from a 0.2% (w:v) solution of KH_2PO_4 and a 0.4% (w:v) solution of $Na_2HPO_4.7H_2O$ in distilled water, mixed to give the required pH. The stain was made up in a stock solution from 3.8 g Giemsa's stain (British Drug Houses Ltd.), 250 g glycerin, and 250 g methyl alcohol. For staining purposes 5 ml of this solution was mixed with 50 ml buffer. Stained preparations were examined using a Cooke, Troughton and Simms research microscope fitted with a 2.2-mm apochromatic oil immersion objective (N.A. 1.32, 80×), a magnification changer (1.5×, and 2.25×), compensating eyepieces (8×), an oil immersion condenser (N.A. 1.4), and a green substage filter (546 mμ). The light source was a multiple-filament lamp controlled by a rheostat (45 watts maximum). An extensible bellows type camera (film size $3\frac{1}{4}\times4\frac{1}{4}$ in.) from the same manufacturer was used for taking photomicrographs. Kodak Contrast Process Panchromatic sheet film was used and developed in Kodak D11 developer. Photographs were taken at a magnification of 1524× or 4480× on the film and were suitably enlarged or reduced to give the magnifications in Plate I.

Results

The appearance of the interphase nuclei, stained according to the method described above, varies to some extent in intensity of staining, shape, size,

FIG. 1. Resting nuclei in old mycelium from still culture, ×1524.
FIG. 2. Clamp-connection formation and attenuated nuclei in young mycelium, ×1524.
FIG. 3. Two nuclei in late prophase; in the nucleus on the left the four chromosomes are well separated; ×1524.
FIG. 4. Two pairs of nuclei, showing a metaphase plate in side view at the lower left and early anaphase in the lower nucleus of the upper pair, ×1524.
FIG. 5. Spindle at late metaphase, ×1524.
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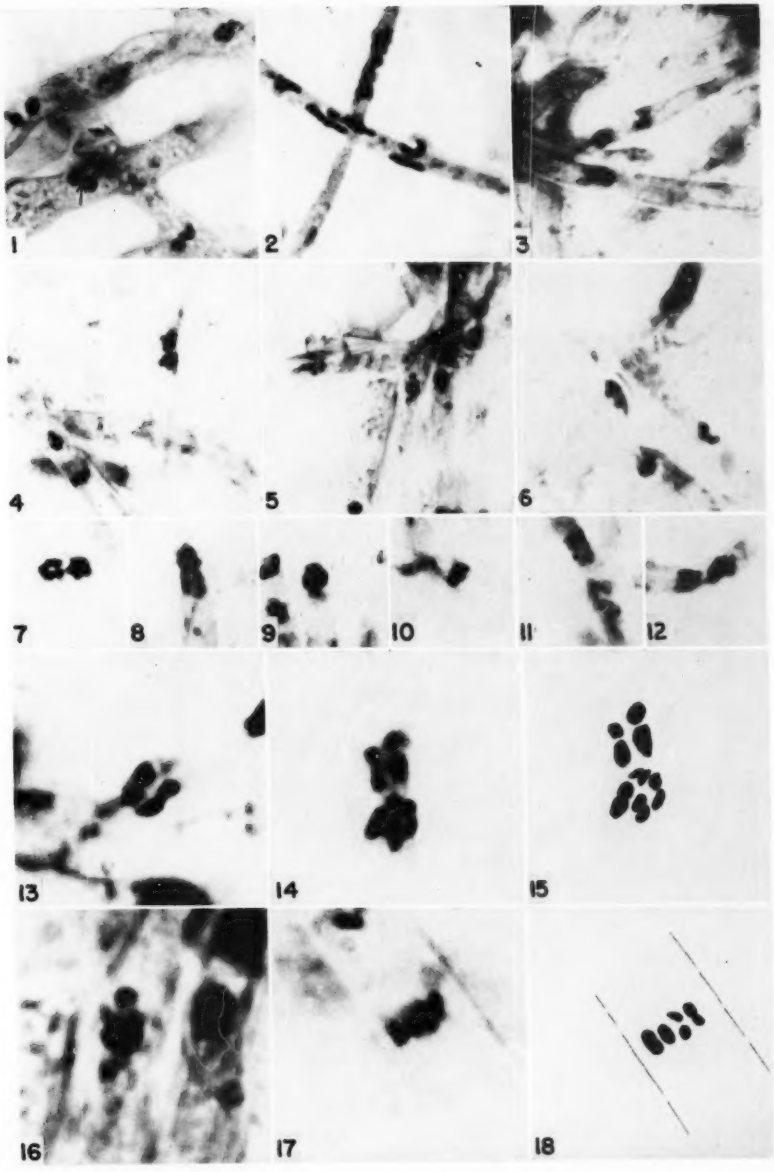
FIG. 7. Late prophase nuclei, ×2240.
FIG. 8. Late prophase nuclei, ×2240.
FIG. 9. Metaphase plate, end view; note width of chromosomes; ×2240.
FIG. 10. Metaphase plate, side view; ×2240.
FIG. 11. Two anaphase nuclei; in the upper nucleus eight chromatids can be distinguished; ×2240.
FIG. 12. Two late anaphase nuclei, ×2240.
FIG. 13. The four prophase chromosomes shown in Fig. 3 photographed at higher magnification, ×4480.
FIG. 14. The upper pair of nuclei shown in Fig. 3, photographed at higher magnification; the lower nucleus can be clearly seen to be in early anaphase; ×4480.
FIG. 15. Diagram of nuclei in Fig. 14, showing arrangement of chromatids in the lower nucleus.
FIG. 16. The metaphase spindle shown in Fig. 5 photographed at higher magnification, ×4480.
FIG. 17. The metaphase plate shown in side view in Fig. 4 photographed at higher magnification, ×4480.
FIG. 18. Diagram of metaphase plate in Fig. 17.

PLATE I



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and degree of association within the cell. Some of these variations may be due to small uncontrolled differences in fixing, hydrolyzing, or staining, but many of them appear to be related to the physiological state of the mycelium. In the older mycelium from still cultures there are usually two nuclei per cell. They are typically closely paired, densely staining bodies, round or oval but occasionally angular or crescent-shaped (Fig. 1). They are usually fairly similar in size, with an average diameter of approximately 1.0 μ. A small stained granule can sometimes be seen lying in the cytoplasm just outside the nucleus (see arrow in Fig. 1). Such nuclei may be described as truly *resting* and are probably unlikely to divide again unless a change in the cultural conditions occurs. In younger hyphae the nuclei stain less densely and are quite variable in shape and position. Sometimes the nuclei become quite attenuated (Fig. 2) and in some cases it appears that this condition is associated with movement of nuclei along the hyphae. "In-line" as well as side-by-side conjugation is readily observed and in the former arrangement the nuclei may be separated by an appreciable distance. The number of nuclei per cell remains typically two although in hyphal tips four nuclei may be present temporarily owing to a lag in septum formation.

Division of conjugate nuclei is often associated with clamp-connection formation, but there are frequent exceptions to this. Even within a single hypha, variation in this respect may occur between successive divisions. The sequence of events in clamp-connection formation appears to be quite typical. One of the two conjugate nuclei enters a short, curved hyphal branch (Fig. 2), the two nuclei divide, and septa are formed in the usual manner.

Nuclear division was most readily observed in mycelium grown in shake culture, when clamp connections were absent, when the nuclei were not attenuated, and when the nuclei, if closely paired, were in an "in-line" arrangement. Division of the paired nuclei usually occurs simultaneously but their orientation is often quite different and appears to be random. This is illustrated by both pairs of nuclei in Fig. 4, and in the nuclei of Figs. 3, 7, 11, and 12. In many cases the two dividing nuclei are so closely associated that they appear as one large complex which it is almost impossible to interpret.

The first clear indication of the onset of mitosis is the appearance of four discretely stained chromosomes in the nucleus (Figs. 3, 7, and 8). No earlier stage than this, distinguishable from the interphase nucleus, can be recognized with certainty and it must be regarded as late prophase. Only when the nucleus is favorably orientated with relation to the light path can all four chromosomes be seen and frequently two or more of the chromosomes may be so close together as to appear united into a bar of chromatin (Fig. 7). This is believed to be due partly to the retention of a certain amount of stain by the matrix surrounding the chromosomes in a manner similar to that described by King (10) in desmid nuclei. Sometimes only three chromosomes are visible as in the lower nucleus of Fig. 8 (four chromosomes can be distinguished in the upper nucleus in the actual preparation) but this is presumed to be due to the orientation. As can be seen from several of the figures, the chromosomes are of different sizes. One is larger than the others and measures approximately 1×0.45 μ. The smallest measures 0.7×0.35 μ. The nuclei at this stage are larger than at interphase and average 1.5–2.0 μ in optical section.

At metaphase most nuclei measure 2.0 μ across their maximum diameter, and the chromosomes show an appreciable increase in width (Fig. 9). At this and earlier stages the chromosomes are most distinctly seen from the end position, but subsequent stages of division are best followed from a partly lateral aspect, although here resolution of the chromosomes is frequently lost. Spindles can be seen in some preparations, and observation of later stages of division is suggestive of the separation of the chromatids towards spindle poles (Figs. 5, 6, 12, and 16). However, it was not possible to study the spindle in detail and chromosome definition was usually poor in preparations where spindles were most readily seen. Metaphase plates were frequently seen laterally and occasionally all the chromosomes can be distinguished in this view (Figs. 4, 17, and 18). In many cases, however, only three (Fig. 10) or even fewer chromosomes can be seen, and in somewhat overstained preparations they appear as a single homogeneous mass (Figs. 5 and 16). Figure 17 shows the metaphase plate in Fig. 4 at high magnification and an explanatory diagram of this is shown in Fig. 18. The four chromosomes can be clearly seen here and the separation of the chromatids of two of the chromosomes is just detectable.

In Fig. 14 the upper pair of nuclei shown in Fig. 4 are magnified 4480×, and they are illustrated diagrammatically in Fig. 15. Both nuclei are at early anaphase but they differ in orientation. Details of division cannot be distinguished in the upper nucleus but in the lower nucleus division is occurring at an angle of approximately 45° to the plane of the slide and in a NE.–SW. direction. The separation of eight daughter chromatids can be clearly seen in this figure and the characteristic differences in chromosome size mentioned earlier are particularly well illustrated. Clear anaphase figures in which eight completely separated chromatids can be distinguished are difficult to illustrate. If the chromatids, which are much smaller than the metaphase chromosomes (compare, e.g., Figs. 9 and 11), are sufficiently separated the proximity of the conjugate nuclei often leads to confusion. Anaphase figures with up to 16 chromatids have been observed under such circumstances. However, because of the disposition of the chromatids at different focal levels, and the presence of a lightly stained matrix between them, definition in photographs of these figures is usually poor. Whether the two nuclei do actually become involved with one another cannot be stated with certainty, but in view of the genetical demonstration of mitotic recombination in fungi (13) the possibility should not be overlooked. Even where nuclei do not overlap, anaphase figures are sometimes difficult to interpret, as, for example, in the lower nucleus in Fig. 11. Only when the orientation is especially favorable can eight chromatids be clearly distinguished at this stage as in the upper nucleus of Fig. 11. The observation of a large number of metaphase and anaphase figures leaves no doubt that eight daughter chromatids arise from the four metaphase chromosomes and that these separate regularly in two groups of four to opposite poles of the spindle.

At the later stages of anaphase and telophase, separate chromatids can no longer be resolved (Figs. 6 and 12). This is presumably due to their small size and proximity as they are gathered towards the spindle poles.

Discussion

The demonstration of clamp-connection formation and the presence of two closely associated nuclei in each cell supports the view that the fungus studied in this investigation is a basidiomycete. The demonstration of a haploid chromosome number of 4 is also in reasonable conformity with this assumption as this is the number most frequently found in the Hymenomycetes (12).

The sequence of events during nuclear division was typically mitotic. This conclusion is based upon the following critical evidence: (1) the appearance of four distinct chromosomes at prophase; (2) the establishment of the chromosomes on a metaphase plate and simultaneous increase in chromosome thickness; (3) the demonstration of spindles; (4) the division of the individual chromosomes into chromatids; (5) the anaphase separation of the members of each chromatid pair to opposite poles of the spindle. Certain details still require further study. For example, the process of spindle formation, and the possibility that the small granule associated with many nuclei (Fig. 1) is a centrosome, have not been examined in detail. Additional information concerning the earliest stages of division is also required. When the chromosomes are first discernible they appear to be fully contracted and approximately the same size as the anaphase chromatids.

The mode of division of somatic nuclei in fungi has been the subject of several recent publications. McGinnis (11) and Somers *et al.* (18) demonstrated individual chromosomes and at least some of the stages of mitosis in *Puccinia graminis* and *Neurospora crassa* respectively. Robinow (14, 15), however, described nuclear division in the *Mucorales*, and on the basis of his observations and lack of decisive evidence of classical mitosis in the published findings of others concluded that division occurred by constriction of the nucleus without the formation of mitotic figures or the appearance of individually distinct chromosomes. Support for these conclusions has been provided by Bakerspigel (1–7) from studies of a variety of fungi including a basidiomycete, *Schizophyllum commune* (5), and in part by Dowding and Weijer (8) and by Turian and Cantino (19). Such conclusions appear to rest largely on the failure to demonstrate typical mitosis rather than on the observation of a clearly defined alternative mode of division. Even without the positive evidence provided in the present paper and by others (11, 18) it seems premature to deny the occurrence of somatic mitosis. Cytological studies of fungi are attended by appreciable technical difficulties, attributable mainly to the small size of the nuclei, and there is much circumstantial evidence that is compatible with the view that mitosis does occur in somatic hyphae.

The methods described above are those which seemed to give most satisfactory results and were chosen after modifications of most steps of the procedure had been tested. However, in spite of this and although the fungus material was homogeneous, differences in the quality of a preparation invariably occurred, even on a single slide. These differences can only be explained by factors such as unequal drying of all parts of the mycelial film and consequent differences in the uptake of fixative and the degree of hydrolysis and staining. The methods successfully used by Hrushovetz (9) to stain ascus nuclei in *Cochliobolus sativus* had to be modified in several details before

satisfactory preparations could be obtained in the present investigation. It seems likely, therefore, that small differences in technique may profoundly affect the appearance of stained nuclei and also that a procedure which gives good results with one fungus will not be completely satisfactory for others. For these reasons comparison of the staining procedure used here with that of other investigators may not explain their failure to demonstrate mitotic figures. However, optimum staining with Giemsa's stain was achieved in 25 minutes in the present investigation, and the use of much longer periods described by other investigators (1, 14) was found to give overstained preparations in which neither individual chromosomes nor stages of division could be distinguished.

The main difficulty in studying fungus nuclei by means of the light microscope is their small size. With the equipment used here the theoretical limit of resolution is approximately 0.21 μ (16). To be distinguished as separate entities, therefore, the chromosomes in a nucleus must be at least this distance apart. The nuclei which have been described here seldom measured more than 2.0 μ in diameter at metaphase and the four chromosomes varied in size from 0.7×0.35 μ to 1.0×0.45 μ. It is, therefore, likely that in many such nuclei chromosomes will be within a distance of 0.21 μ of each other. Even when the chromosomes are sufficiently separated only a proportion of nuclei are likely to be suitably orientated in relation to the light path to permit differentiation. The combination of these difficulties of resolution with unsatisfactory fixing and staining may account for many of the failures in attempts to demonstrate mitotic division in the somatic nuclei of fungi.

In view of these considerations, circumstantial evidence of somatic mitosis cannot be lightly dismissed. Olive (12) has summarized many publications which clearly indicate that in the process of sexual reproduction in a wide variety of fungi meiotic and mitotic nuclear divisions occur that are essentially similar to those in higher organisms. To classify the final mitotic divisions in the formation of ascospores in *Neurospora* spp., for example, as sexual and hence different from nuclear divisions in the vegetative hyphae, as suggested by Bakerspigel (3), and Dowding and Weijer (8), does not appear to be entirely reasonable. Particularly strong evidence of the presence of chromosomes in somatic nuclei, and of the occurrence of nuclear division by mitosis, is provided by the demonstrations of mitotic recombination in fungi, summarized by Pontecorvo and Käfer (13).

The demonstration of somatic mitosis in a basidiomycete, as described in this paper, does not eliminate the possibility that other forms of somatic nuclear division may occur in the fungi. It does suggest, however, particularly in view of the other considerations which have been discussed, and of the genetical implications of amitosis, that a most cautious attitude be adopted towards reports of amitotic nuclear division in somatic hyphae.

Acknowledgments

The authors wish to express their thanks to Mr. B. D. Atkins for valuable advice on the use of equipment and to Cooke, Troughton and Simms Ltd. for the microscope camera, which was so generously provided during the course of these investigations.

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MICROFUNGI

VIII. BALANIUM WALLROTH[1]

S. J. HUGHES AND G. L. HENNEBERT[2]

Abstract

Revised diagnoses of the monotypic genus *Balanium* Wallroth, and its type species *B. stygium* Wallr., are provided from an examination of the type collection and more recent collections. The fungus is illustrated.

In 1833 Wallroth described his new genus *Balanium* for a single species *B. stygium*. He published no illustration and for more than a hundred years the fungus has not been understood.

Reichenbach (1841) listed the generic name. Corda (1842*a*, 1842*b*) included Wallroth's diagnosis of the generic name and added 'Zur Verdeutlichtung der Gattung wäre eine gute Abbildung dringend nothwendig.' Rabenhorst (1844) included an abbreviated more-or-less word-for-word translation of Wallroth's diagnosis. Léveillé (1846) listed *Balanium* Wallr. with *Menispora* Pers. and other generic names in his tribe 'Menisporés'. In the account of the 'Fungales', prepared by Berkeley for Lindley's (1846, 1847) *Vegetable Kingdom*, *Balanium* was included with the 'genera not sufficiently known.' Saccardo (1886) compiled the generic name with 'genera dubia vel excludenda.' In the fourth edition of the *Dictionary of the Fungi* by Ainsworth and Bisby (1954) *Balanium* was included as '?Fungi' but in the fifth edition (Ainsworth 1961) as 'Moniliales . . . fide Hughes [1958].' Ellis (1961) redescribed and illustrated the single species.

The type collection is preserved in Wallroth's herbarium in the University of Strasbourg and was examined by one of us in 1955. The fungus was immediately reminiscent of a number of collections made in England and preserved in Herb. IMI.[3]

In view of the doubt surrounding the name *Balanium*, the type species *B. stygium* is redescribed and illustrated (Fig. 1 and Plate I, Fig. 3) and a revised generic diagnosis given, based on the type collection and six other collections.

Wallroth described his species as follows: '*B. stygium* W., rhabdis continuis opacis vage-ramosis, extremitatibus dichotomis corpuscula sporomorpha subpyriformia demum secedentia sustinentibus, in stratum nigrum effusum laxe intertextis'.

'Ad ligna putredine exesa sub sepimentis occultata Thuring. (Wallr.) (Rhabdi e basi simplici continui vage-ramosi, in extremitates patentes dichotomas, primum corpusculo sporomorpho obovato solitario coronatas desinunt

[1]Manuscript received May 24, 1961.
Contribution No. 152 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.
[2]Postdoctorate Research Fellow from the University of Louvain, Belgium.
[3]Reference to particular herbaria is made by use of abbreviations found in Index Herbariorum (Regnum Vegetabile, Vol. 6 (1956)).

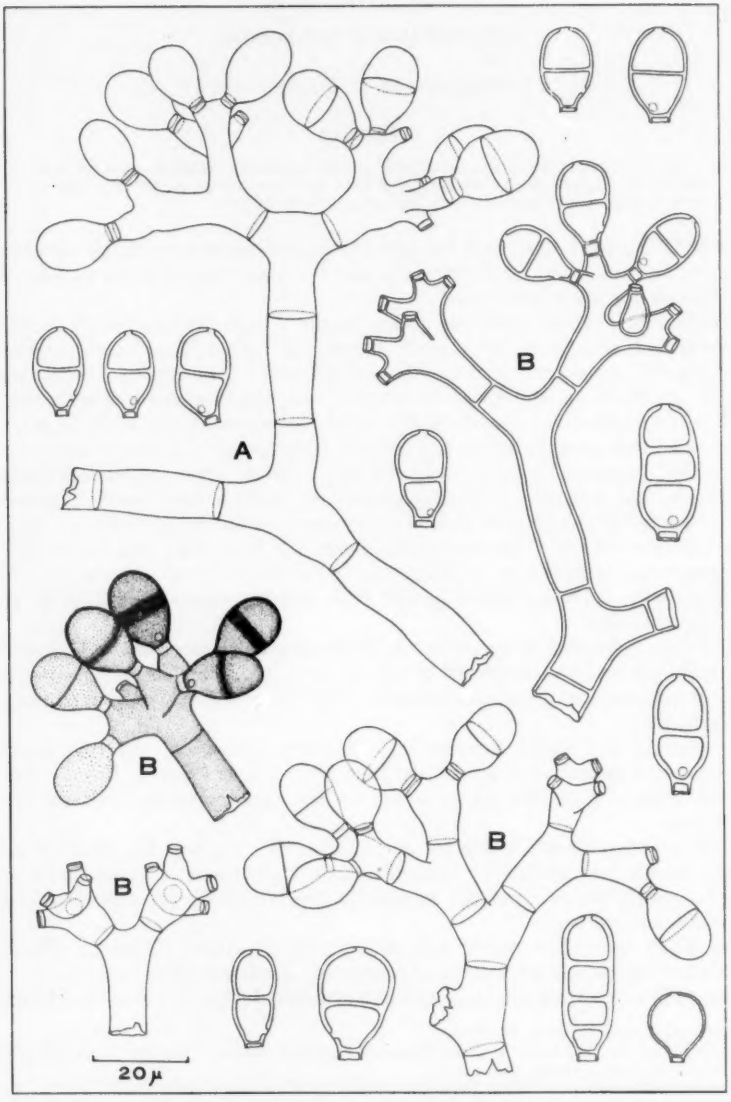
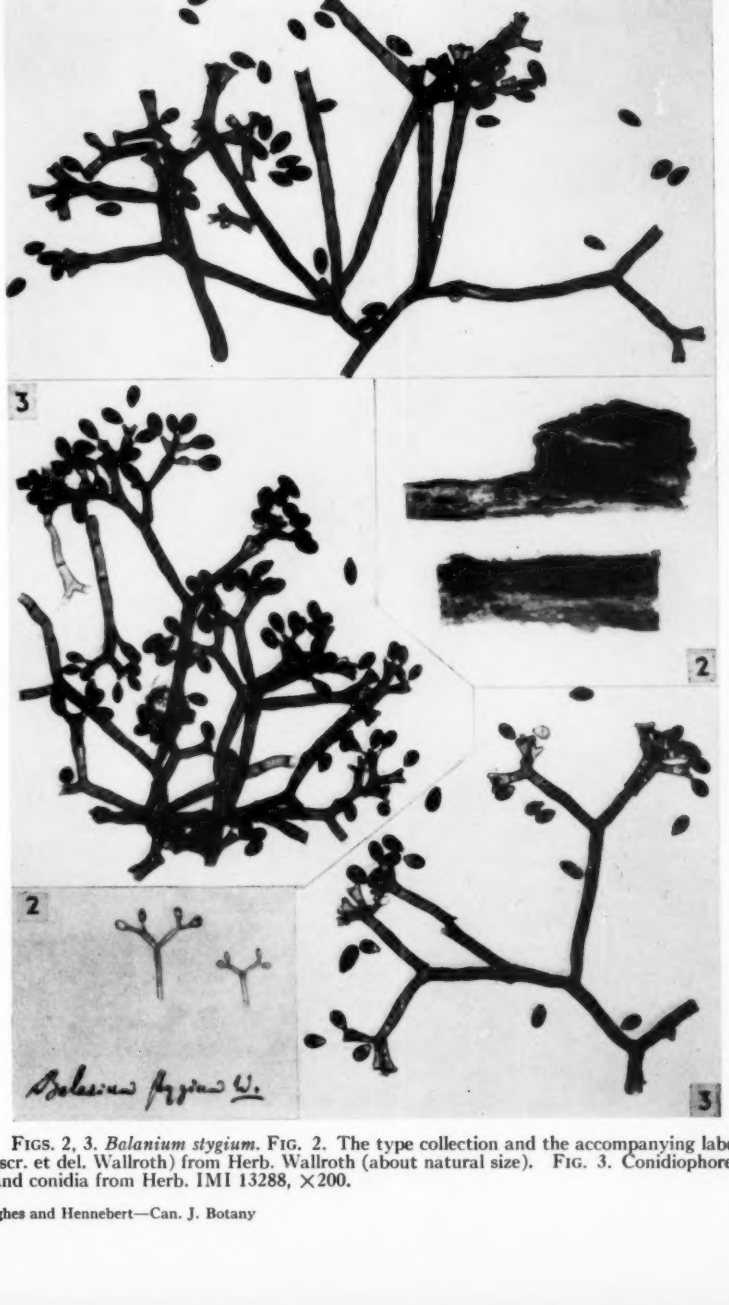


FIG. 1. *Balanium stygium:* A, portion of conidiophore and detached conidia from the type collection; B, portions of conidiophores from collection No. 2. ×750.



FIGS. 2, 3. *Balanium stygium.* FIG. 2. The type collection and the accompanying label (scr. et del. Wallroth) from Herb. Wallroth (about natural size). FIG. 3. Conidiophores and conidia from Herb. IMI 13288, ×200.

illisque basi pedicelliformi facile fracta, undique dispersis insperguntur, et cubilia strati fibrosi stygii parce inquinantis instar, longe lateque obducunt.)'

There are two features of the diagnosis which have undoubtedly resulted in the fungus not again being recognized under this name. In the first place the conidia ('corpuscula sporomorpha subpyriformia') were not described as 1-septate, although they are mostly 1-septate in the type and in more-recent collections. In the second place, the phrase 'rhabdis continuis' appears in both generic and species diagnoses, suggesting nonseptate mycelium. However, in the 'clavis systematica' (p. xv) *Balanium* was included by Wallroth in that category characterized by 'rh[abdis]. septatis'. It may be added that in dried material, air bubbles are very difficult to remove from the dark conidia and conidiophores and obscure the septa.

Redescription of *Balanium* Wallroth

Dematiaceous saprophytic hyphomycetes.

Conidiophores solitary or in small groups, septate, wide, dark brown, dichotomously branched, terminating in short, simple or branched bicuspid or tricuspid (2–3 denticulate) sporogenous cells.

Conidia produced singly as blown-out ends of denticles of sporogenous cells from which they are nearly always delimited by a separating cell. The conidia are oval to pyriform, generally 2-celled, thick-walled, dark brown, smooth and dry.

Redescription of *Balanium stygium* Wallroth

Colonies black effuse, up to 30 mm wide, composed of sparse or densely crowded arborescent conidiophores 1 to 2 mm high.

Mycelium immersed, composed of branched septate pale brown to dark brown hyphae 1.5–11 μ wide which show occasional anastomoses and here and there form small aggregations of cells.

Conidiophores usually solitary, sometimes in groups of two or three; they are regularly septate, brown to dark brown, thick-walled (ca. 0.7 μ), smooth, more or less cylindrical and 7–10 μ wide throughout. The basal unbranched part of the conidiophore is straight and erect and 200–500 μ long: it is then repeatedly (up to 7 times) dichotomously branched (at 80°–90°) with the branches decussate to the supporting branch. The ultimate cells of the branched conidiophore develop into sporogenous cells. These may be bicuspid or tricuspid, bearing two or three divergent, usually subulate denticles. Alternatively a sporogenous cell may itself be branched so that it is composed of two biscuspid or tricuspid elements.

Conidia are produced singly as blown-out ends on the denticles of sporogenous cells and form rosettes of 6–12 conidia. They are nearly always delimited from the sporogenous cell by a disk-shaped, hyaline to subhyaline separating cell 4–8 μ long and 1–2 μ wide. Mature conidia are oval to pyriform, generally two-celled, rarely one-, three-, or four-celled, thick-walled (ca. 1 μ), dark brown to black, smooth, and dry. A broad transverse black zone often develops and obscures the septum. At the apex of the conidium and usually just above the base as well, there are thin-walled circular areas which may be germ pores. At the base, fallen conidia bear a hyaline to subhyaline frill resulting from the

irregular fracture of the separating cell. Conidia measure (13)15.5–21.5 (25) × 8–14 μ, averaging 18×11 μ.

Habitat: on rotten wood of *Sambucus nigra* (England) and on unidentified wood (Germany).

Collections examined: Type collection and presumed parts of the type. (1) '*Balanium stygium* W. [scr. Wallroth],' in Herb. Wallroth in Herb. STR. This specimen consists of two pieces of rotten wood bearing an effuse black colony of conidiophores. Accompanying the specimen is a sketch (Fig. 2), presumably by Wallroth, which shows the 'rhabdis continuis . . . . dichotomis corpuscula sporomorpha subpyriformia' of his diagnosis. (2, 3) In the folder next to that containing the above collection in Herb. Wallroth are two sheets, both unlabelled, and bearing *B. stygium* on pieces of rotten wood. These may well be part of the collection (1) described above.

Other collections: On rotten wood of *Sambucus nigra*: (4, 5) Ham Common, Surrey, England, 5.IV.1947, IMI 13288, 13314(*b*) (E. W. Mason and S. J. H.); (6) 4.VII.1948, IMI 29515 (S.J.H.); (7, 8) Howldale, Yorks., England, 13.IV.1947, IMI 13907, 13908 (E. W. Mason and S. J. H.); (9) Bolton Percy, Yorks., England, 20.XI. 1948, IMI 33207 (W. G. Bramley).

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INDUCED VARIATION IN FUSARIUM OXYSPORUM[1]

O. T. PAGE

Abstract

A *Fusarium* isolate produced polygalacturonase (PG) of the endo or I type. Pectic acid was hydrolyzed at random liberating intermediate oligogalacturonic acid residues which were separated chromatographically. The formation of PG in the presence of a specific carbon source was shown to be an induced synthesis. It was also shown that sucrose was inhibitory of production but not activity of PG. Culture techniques precluded mutation and selection as significant factors in the induced formation of PG. The similarity of induced enzyme synthesis in *Fusarium* and certain bacterial enzymes is discussed and the significance of the work as related to wilt mechanisms is considered.

Introduction

Although variation has long been recognized in *Fusarium oxysporum* Schl. (23), relatively little is known of the mechanisms underlying this variation. Buxton (2) has established that heterokaryosis and parasexuality are genetic recombination phenomena in *Fusarium* and has proposed (3) that 'adaptation' may also be a mechanism of variation. It has not been established, however, whether 'adaptation' is attained by the selection of variants from a genotypically heterogeneous population, or whether 'adaptation' can occur in a genetically homogenous cell population. This paper reports experiments to determine whether enzyme formation, as one aspect of 'adaptation', can be induced in a homogenous population of *Fusarium* cells.

It is essential to clarify the use of the terms 'adaptation' and 'enzyme induction'. 'Adaptation' describes all of the various processes of change which confer on an organism fitness to its environment (25). Modifications in enzymatic constitution which increase fitness would be 'enzymatic adaptations', whether or not they were primarily mutational. 'Enzyme induction', as used here, denotes a relative increase in the rate of synthesis of an enzyme following exposure of cells of constant genotype to a chemical inducer (5).

The pectic enzyme polygalacturonase (PG) was selected to investigate induced enzymatic variation in *F. oxysporum* because of its 'adaptive' nature (20), and because of its potential significance in wilt diseases of plants (27), particularly as related to vascular plugging (9). PG is one of two principal groups of pectic enzymes (pectinases), of which the pectinesterases (PE) de-esterify pectin and pectinic acid while the PGs hydrolyze, either randomly or terminally, the α-1,4′-glycosidic linkages of demethylated polygalacturonic (pectic) acid (6, 8).

Methods and Results

Nature of Fusarium Polygalacturonase

Carbohydrates to be tested as PG inducers were selected after preliminary studies in which *F. oxysporum* was grown on substrates reported to be either

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Contribution from the Central Research Laboratories, United Fruit Company, Norwood, Massachusetts, U. S. A.

'excellent' or 'absent' with respect to pectinase production by *Penicillium chrysogenum* (20). A method for quantitative paper chromatographic assay of products of PG activity in microcultures of *Fusarium* has been described previously (18).

An isolate of *F. oxysporum* was obtained from a pseudostem tracheary element of a Gros Michel banana plant showing *Fusarium*-wilt symptoms. The technique for isolating *Fusarium* from individual host elements has been described previously (17). Microconidial suspensions from single-spore subcultures maintained on 2% water agar were used as inoculum in all experiments. A solution containing NH_4NO_3 (2.0 g), KH_2PO_4 (0.5 g), and $MgSO_4.7H_2O$ (0.5 g per liter) was used as a basal medium.

Enzyme preparations for the chromatographic identification of PG by the method of Reid (22) were obtained from cultures grown on the basal salt solution containing 0.4% D-galacturonic acid. After adjustment of the medium to pH 4.5 with dilute KOH, 50-ml De Long flasks were charged with 10-ml aliquots and autoclaved 10 minutes. Following inoculation with a spore suspension, cultures were incubated at 27° C for 7 days and the contents of 40 flasks were combined, homogenized, and filtered. The filtrate was precipitated with $(NH_4)_2SO_4$ (12), dialyzed in an Oxford Multiple Dializer against HCl–phthalate, pH 3.7, and freeze-dried. Enzyme preparation was carried out in a cold room at a temperature of 4° C.

This purified preparation at 0.5% in pH 3.7 HCl–phthalate buffer was combined with 0.5% Mann-assayed polygalacturonic acid and incubated at 33° C for 16 hours. Hydrolysis of polygalacturonic acid by the PG preparation was indicated by chromatographic identification of the end product, monogalacturonic acid (18).

Fusarium enzyme preparations were compared chromatographically with commercial enzyme samples (Nutritional Biochemical Corp.). In first trials, 0.5% commercial pectinase and PE in citrate–phosphate buffer, pH 3.6, were applied to Whatman 3 mm filter paper in 40–100 μl aliquots. Chromatograms were developed at a temperature of 4° C by ascending irrigation with either 0.33 *M* NaCl (pH 5.8) or citrate–phosphate buffer at a pH range of 2.7–7.0. The chromatograms were hung briefly after development to remove excess water, and while still moist were placed on a 3–4 mm layer of 1% sodium ammonium pectate solidified with 1.5% agar in Lucite plates, 34×14×1.5 cm, fitted with a tight cover. The agar plates were variously buffered from pH 4.0 to 7.0; below pH 4.0 difficulty was encountered in solidifying the agar. After incubation at 33° C for 18 hours the plates were flooded with 0.05% bromophenol blue in ethanol to differentially color zones of hydrolysis.

The best separation and development of pectolytically active zones occurred with solvent at pH 3.0 and substrate at pH 4.0–5.0. Two convergent zones of activity (Fig. 1A), similar to zones for PG illustrated by Reid (22), were observed when a commercial pectinase preparation was irrigated with 0.33 *M* NaCl and subsequently assayed on pectate agar at pH 4.0. When the same amount (40 μl of 0.5% pectinase) was chromatographed with citrate–phosphate solvent, a single broad zone was observed on the assay plate (Fig. 1B, P). The zone of pectate hydrolysis with a commercial PE preparation, Fig. 1B,

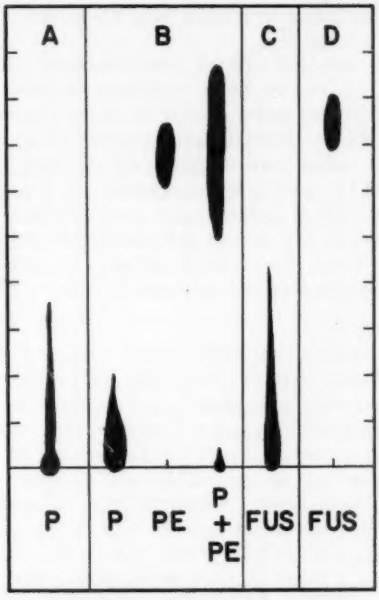

FIG. 1. Separation of pectolytically active zones in commercial pectinase (P) and pectinesterase (PE), and in a *Fusarium* (FUS) enzyme preparation. Chromatogram A was irrigated with 0.33 *M* NaCl; B and C with citrate–phosphate buffer, pH 3.0; and D with citrate–phosphate buffer, pH 7.0. All chromatograms were assayed on pectate agar at pH 4.0.

PE, is indicative of a different enzyme protein, as judged by the greater movement in the citrate–phosphate solvent. When the same amounts of pectinase and PE were combined (Fig. 1B, P + PE), the zone corresponding to PE was markedly larger than that obtained with PE alone. The zone at the point of origin corresponding to pectinase was considerably smaller than the zone obtained with pectinase alone.

Enzyme preparations from cultures of *Fusarium* were also chromatographed. Two distinct zones of activity were demonstrated. The zone obtained at pH 3.0 (Fig. 1C) agreed with that proposed for PG (22). A second zone (Fig. 1D), which was most distinct at pH 6.0–7.0, corresponded with that obtained with commercial PE. Both zones were detected on essentially demethylated substrate.

The products of polygalacturonic acid hydrolysis by *Fusarium*–PG were next investigated to determine whether the method of attack was random or terminal. Polygalacturonic acid, 0.5%, was added directly to 7-day-old cultures grown on a three-salt solution containing 0.02 *M* mucic acid as the carbon source. The pH of the culture – polygalacturonic acid mixture was adjusted to pH 4.0, and it was then incubated at 37° C. After 1 hour and 16 hours the hydrolytic products were determined by chromatographing 200-μl

filtrate samples by ascending irrigation with an acidified ethanolic colorizing solvent (18).

Seven spots were detected after 1 hour whereas only monogalacturonic was apparent after 16 hours. Such multispot sequences were interpreted as an oligogalacturonic acid series similar to those demonstrated for random scission of pectic acid by *Botrytis* and several other fungal PGs (8, 12). The R_f values of the series were: mono- 0.63, di- 0.53, tri- 0.44, tetra- 0.33, penta- 0.23, hexa- 0.15, and >hexa-galacturonic acid 0.08–0.0. Chains of more than six α-1,4′-linked galacturonic acid residues were apparently of sufficient molecular weight to permit only negligible movement during 30-cm travel of the solvent front. The random scission of glycosidic linkages found with *Fusarium*–PG is characteristic of endo-PG (6) or Type I PG (8).

Induction Experiments

Because of the possible composite nature of population 'adaptability' it remains to be established that PG synthesis can be induced in homogeneous populations of *Fusarium* cells exposed to a particular chemical substrate.

All glassware was soaked overnight in dichromate – sulphuric acid, washed in running tap water, autoclaved in 0.1% Versene for 30 minutes, and finally rinsed five times in ion-free water and dried. To obtain uniform cell populations, single microconidia from a parent culture of single-spore origin were transferred aseptically from water agar to 0.25 ml of basal sucrose (0.02 *M*) medium in microcups, 1.1 cm by 1.0 cm (O.D.). Inoculated microcups were placed in sterile 50-mm Petri plates and incubated for 96 hours in a water bath at 27° C. Following the development of cultures on sucrose, microcups were dropped into beakers of sterile distilled water to float the mycelial mats; the mats were washed in three rinses of distilled water. Samples of 10 mats were collected individually on 25-mm HA type Millipore filters, removed from the filters, placed in 50-mm Petri plates to dry for 6 hours at 100° C, and weighed. At least 40 washed mats per experiment were transferred aseptically to microtubes, 4.5 cm by 1.0 cm (O.D.), containing 0.5 ml of either 0.02 *M* sucrose, or mucic acid at an equivalent carbon level, in addition to KH_2PO_4 and $MgSO_4.7H_2O$, each at 0.5 g per liter. Microtubes were placed in covered 50-ml beakers and incubated in a water bath at 27° C for a further 96 hours.

After incubation on either sucrose or mucic acid, without added nitrogen, 0.25 ml of 0.5% polygalacturonic acid in HCl–phthalate buffer was added to each microtube culture. Hydrolysis at 37° C was continued for 15 hours, at which time the dry weights of 10 mats were determined for each carbon substrate. Filtrates were examined chromatographically for the presence of galacturonic acid.

The essential steps in the foregoing sequence were: (1) single-spore cultures were grown for 96 hours on sucrose; (2) mats were washed and transferred to either mucic acid or sucrose and incubated, without added nitrogen, for an additional 96 hours; (3) polygalacturonic acid was added to each culture tube and PG activity determined by assay for galacturonic acid; and (4) mycelial weights were found after growth on sucrose and after PG assay on mucic acid and sucrose.

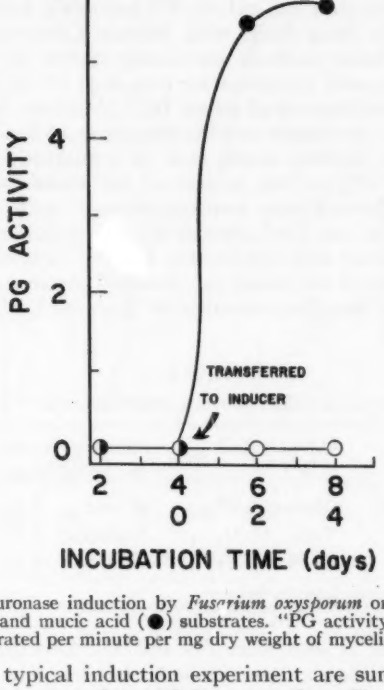

FIG. 2. Polygalacturonase induction by *Fusarium oxysporum* on initial sucrose (◑), control sucrose (○), and mucic acid (●) substrates. "PG activity" refers to the μg of galacturonic acid liberated per minute per mg dry weight of mycelium.

The results of a typical induction experiment are summarized in Fig. 2. Galacturonic acid was not detected in more than 50 single-spore sucrose cultures. However, in cultures grown under identical conditions, but randomly assigned to mucic acid rather than sucrose, relatively high levels of PG activity were detected. A range in PG activity among individual microcultures was estimated to be 1.4–2.6 μg of galacturonic acid per μl of filtrate. This variation was similar to the 'normal' population variation previously demonstrated among single-spore microcultures (17).

During incubation in the absence of added nitrogen 'resting' cell populations gradually lost weight, indicative of cell autolysis. The micromats, with a mean weight of 0.66 mg at the time of transfer, weighed 0.35 and 0.50 mg after 50 hours' incubation on mucic acid and sucrose, respectively. Although autolysis was permitted to continue for 168 hours after transfer of initial culture to sucrose, no PG activity was detected in the absence of the inducer. It was considered therefore that a failure to produce PG, rather than release of endogenous PG autolytically, characterized cultures incubated in sucrose medium.

Despite the observation that mucic acid functions as an inducer of *Fusarium*–PG, the alternative exists that sucrose may prevent PG synthesis. Furthermore, substrate pH may mask the inhibition of PG production or PG activity.

An experiment was therefore designed to examine the interaction of sucrose and mucic acid substrates, and pH, on PG formation and activity.

Fifty-milliliter De Long flasks with Morton Closures were charged with 10-ml aliquots of basal medium containing 0.0075 *M* sucrose, inoculated by spore suspension, and incubated for 6 days at 27° C. Mycelial mats were washed in successive aliquots of either HCl–phthalate (pH 3.6), phthalate–NaOH (pH 5.4), or potassium–sodium phosphate buffers (pH 7.2), and then transferred to either sucrose, mucic acid, or a mixture of sucrose and mucic acid, at a level of 0.1% carbon, in each of the above three buffer solutions. After 4 days' incubation mats were transferred, with suitable washing in HCl–phthalate buffer, into 5-ml aliquots of HCl–phthalate, pH 3.6, containing 0.25% polygalacturonic acid. Incubation at 37° C was continued until polygalacturonic acid could no longer be detected chromatographically in PG-active cultures, and the galacturonic acid liberated by hydrolysis was completely metabolized.

TABLE I

The influence of buffer–substrate combinations on PG production

Substrate	HCl–phthalate (pH 3.6)	Phthalate–NaOH (pH 5.4)	Na–K phosphates (pH 7.2)
Sucrose	Absent (3.6)*	Absent (5.4)	Absent (6.9)
Mucic acid	Trace (3.2)	Complete† (4.9)	Complete† (6.5)
Sucrose + mucic acid	Absent (3.3)	Absent (4.6)	Absent (6.4)

*pH at the conclusion of a 4-day induction period.
†1.6 mg polygalacturonic acid hydrolyzed per mg dry weight of mycelium.

The results (Table I) show that PG was not detected in the presence of sucrose. It was also shown that although the complete hydrolysis of polygalacturonic acid occurred in the HCl–phthalate–polygalacturonic acid suspension at pH 3.6, PG formation was markedly reduced at this pH. Subsequently, it was found that sucrose added to PG-active culture filtrates did not noticeably inhibit PG activity. Since it was thus clear that PG production, rather than activity, was suppressed in the presence of sucrose in non-growing cultures, the influence of sucrose on PG production during growth was next investigated.

Spore suspensions were used to inoculate 10-ml aliquots of basal medium amended with either sucrose (0.0075 *M*), mucic acid (0.015 *M*), or a combination of these two carbon sources at the same molarities. Mats were collected on Millipore filters, transferred to 2-ml amounts of 0.1% polygalacturonic acid, pH 4.0, and incubated for 8 hours at 37° C. Changes in mycelial dry weight and pH during the growth period are presented in Fig. 3.

PG activity was first detected at 96 hours in sucrose – mucic acid cultures and 24 hours later in mucic acid cultures, but not in sucrose cultures. PG activity in sucrose – mucic acid cultures coincided closely with the termination of exponential growth on sucrose and an accompanying shift in pH. The cell mass produced in 96 hours on sucrose – mucic acid was 14 times that produced on mucic acid alone. This difference in fungal growth probably

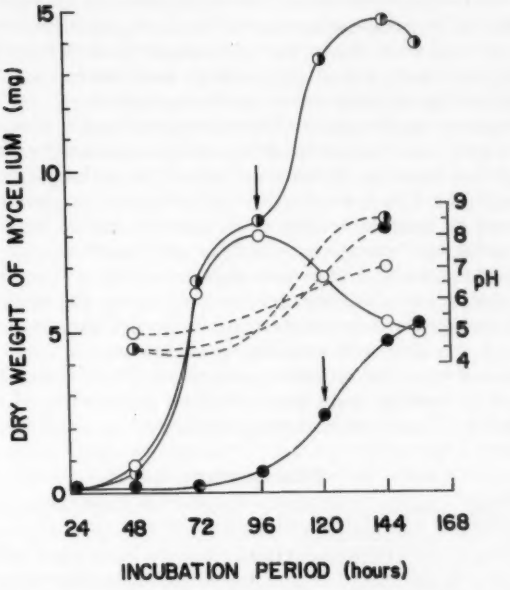

FIG. 3. Mycelial weight (——) and pH changes (– – –) in cultures of *Fusarium oxysporum* grown on 0.0075 *M* sucrose (○), on 0.015 *M* mucic acid (●), and on 0.0075 *M* sucrose and 0.015 *M* mucic acid combined (◑). The arrows indicate the first detection of polygalacturonase activity.

accounts for the detection of PG in sucrose – mucic acid cultures before detection on mucic acid.

F. oxysporum in a state of active growth in the presence of a known PG inducer will not synthesize the level of PG it is capable of producing until the excess sucrose has been utilized. In previous experiments it was also shown that PG synthesis occurred during cell autolysis, in which instance either protein turnover or activation of specific free amino acids presumably provided an adequate source of amino acids for PG synthesis. These findings, which are similar to those observed in certain bacteria (15, 19, 21), indicate that PG synthesis is determined by the carbon environment. To permit an estimate of the time required for synthesis of PG and active hydrolysis of pectic substrate by the newly formed enzyme, a starvation technique was employed.

A spore suspension of *Fusarium* harvested from water agar was used to inoculate 10-ml aliquots of basal medium containing 0.0075 *M* sucrose in 50-ml De Long flasks. After 96 hours' growth at 27° C, mycelial mats were collected individually on sterile Millipore filters, washed with 250 ml of sterile distilled water, and transferred aseptically to 10-ml aliquots of sterile distilled water in De Long flasks. The flasks were placed on a reciprocating

shaker for 40 hours at 27° C to ensure utilization of endogenous sucrose. At the conclusion of the starvation period 25 mg of polygalacturonic acid was then introduced into each flask, the pH adjusted to 4.5 with 5% KOH, and the incubation continued in still culture in a water bath at 30° C. At 2-hour intervals 100-μl samples were chromatographed.

Trace PG activity was detected chromatographically 6 hours after the introduction of polygalacturonic acid into sucrose-depleted cultures whereas no PG activity was found in filtrates incubated for as long as 48 hours with polygalacturonic acid. The low initial rate of polygalacturonic acid hydrolysis by cells depleted of sucrose, under conditions favorable for PG synthesis, was in contrast to the complete hydrolysis of the 25-mg polygalacturonic acid samples within 24 hours after introduction of the polygalacturonic acid. The role of galacturonic acid feedback in accelerating PG synthesis was not investigated, although it was established in preliminary experiments that galacturonic acid was an excellent inducing substrate.

It is to be noted that the *de novo* synthesis of PG within a 6-hour period in a population of 'resting' cells precludes the probability of selection and subsequent growth of some mutant cell population.

Discussion

Because of the possible composite nature of population 'adaptability' Spiegelman (24) has emphasized the necessity of distinguishing experimentally among specific enzyme synthesis induced by an exogenous inducer in: (1) all members of a population of genetically homogeneous cells; (2) certain naturally selected variants possessing the necessary enzyme pattern from a genetically heterogeneous population; and (3) a combination of (1) and (2) in which the enzyme is induced by the substrate in certain variants and these are subsequently selected. Nonseptate spores of *F. oxysporum* are known to be not only haploid but also predominantly uninucleate (1). Since parasexual recombination is an event of extremely rare occurrence (2), genetic homogeneity may be essentially assured by several successive single sporings of a parent culture. PG was produced by many replicate single-spore cultures of common single-spore origin only in the presence of an inducing carbon source. Therefore, genetic heterogeneity could not have been a factor in PG induction. Furthermore, the appearance of PG in less than 6 hours precludes preferential development of a mutant cell population producing a relatively high constitutive level of PG.

Fusarium cells produced PG on inducing substrates but cells of the same genotype were unable to synthesize detectable levels of PG on sucrose. Alternative regulatory mechanisms, i.e. the induction versus the repression of enzyme formation, have also been demonstrated in other microorganisms. For example, excess hexose repressed β-galactosidase synthesis in *Escherichia coli* and this inhibition was suppressed only when excess of hexose was metabolized (7). Similarly, cellobiose may inhibit production of cellulase in rapidly growing cultures of *Trichoderma viride* even though cellobiose is an inducer of cellulase. A marked increase in cellulase production occurred within a temperature range which was suboptimal with respect to growth of *T. viride*

(14). Halvorson (11) in a recent review has considered that a decision between repression and induction awaits a critical examination of induction in cell-free systems. The present study supports the 'unitary' concept (4).

The results of Mandelstam (15) are pertinent to the present discussion. When *E. coli* was grown on a medium containing glucose and lactose, growth took place in two phases in the first of which cells grew at the expense of glucose. After exhaustion of glucose the cells entered a diauxic lag possessing only traces of β-galactosidase. The cessation of growth during the lag phase initiated synthesis of more enzyme. Synthesis was envisioned to be either preferential, and at the expense of a very small free amino acid pool, or, a more likely alternative, due to the breakdown of proteins to free amino acids, a proportion of which was then used for the *de novo* synthesis of β-galactosidase. Mandelstam also considered it likely that the lag period encountered in the transfer of cells from a rich to a simpler medium was probably characterized by enzyme synthesis from turnover material.

A similar pattern of biochemical differentiation was apparent when *Fusarium* was grown on a medium containing sucrose and mucic acid or was transferred from an initial sucrose medium to a PG-inducing substrate. Since precautions, such as repeated washing of mycelial mats followed by 48-hour starvation in distilled water, were taken to exclude exogenous nitrogen, PG synthesis must have been at the expense of some endogenous nitrogen source such as the free amino acids. The initial detection of PG during the lag phase, after sucrose utilization in a medium containing sucrose and mucic acid, or after transfer with washing to an inducing substrate, coincided with the period during which protein breakdown might be expected. Pollock (21) has suggested that there may be some as yet unknown controlling mechanism for the determination of priorities between different enzyme-forming systems for the utilization of energy and building blocks necessary in enzyme synthesis.

One of the major obstacles encountered in the present investigation was the measurement of PG activity. Wood (27) has pointed out that all too frequently PG activity has been measured only in terms of viscosity changes which can be misleading with respect to the enzyme being studied. The method of choice now applied in estimating PG activity is to measure increase in reducing power during hydrolysis of pectic substrates. The limitations of this and other methods have been discussed by Kertesz (13). Measurement of PG activity by quantitative paper chromatography of galacturonic acid liberated (18) also has certain limitations. For example, spot area distortions resulted from the proximity of buffer salt spots. Although such distortion was overcome by the use of culture transfers in distilled water, the measurement of variations in the color intensity of spots was not resolved satisfactorily. However, chromatography does have the distinct advantages of permitting not only the detection of <20 μg galacturonic acid but also the separation of the end products of PG hydrolysis.

The role of carbohydrate substrates in PG induction and/or inhibition has generally been overlooked in studies of mechanisms of wilting by pathogenic *Fusaria*. The presence or absence of PG is critical to an understanding of wilting mechanisms in plants infected with *F. oxysporum*. For example,

a mutant strain of *F. oxysporum* f. *lycopersici* which did not produce pectolytic enzymes, e.g. PG, was only moderately pathogenic to tomatoes in contrast to the wild type (16). Waggoner and Dimond (26) proposed that plugging of vessels by "maceration" of tomato xylem was dependent upon the presence of *Fusarium*–PG. It was considered highly probable that xylem "maceration" contributed to pathogenesis despite the fact that PG was not produced on glucose and sugars are commonly found in vascular sap. These investigators did not detect PG in diseased plant sap. On the other hand, Gothoskar *et al.* (10) have stressed the significance of an absence of PG which, if present, would break down the pectin of the host cell wall into small soluble fragments which might not be able to cause plugging of vessels of *Fusarium*-infected tomato plants. Clearly the role of PG in the *Fusarium*-wilt syndrome requires further elucidation, particularly with respect to its synthesis *in vivo*.

Buxton (3) has emphasized that caution is necessary in interpreting basic mechanisms until more is known of the influence of 'adaptation' by *in vitro* studies and that many of the problems of variation in pathogenic fungi, including *Fusaria*, are not easy to explain by any other mechanism than 'adaptation'—taken in its general sense. Generally, little thought is given to the influence of artificial substrates on which a pathogen is grown prior to use as inoculum. In view of a demonstrable diauxic phenomenon it cannot be assumed that *Fusarium* cultured as an inoculum source on a complex decoction medium will have the same enzymic phenotype as one grown on different carbon or nitrogen substrates, and then combined as inoculum.

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STUDIES OF THE CULTURE OF ATROPELLIS PINIPHILA[1]

J. C. HOPKINS[2]

Abstract

Isolates of *Atropellis piniphila* were grown from single ascospores, and from cankers on lodgepole pine. A variety of media were tested for their capacity to support growth of the isolates. Descriptions of cultural and microscopical characters were prepared. Endoconidia were produced abundantly by most of the isolates after growth at 50%, or higher, relative humidity, but they were absent from most of the cultures which had been grown at 10% relative humidity. Attempts to grow apothecia in culture were unsuccessful. The cardinal temperatures for growth occurred at 4°, 18°, and at 24° C. The optimum pH for growth in buffered media occurred at between 3.0 and 4.0. In media containing varying concentrations of carbon and nitrogen, growth was maximal on those containing 4% dextrose and 0.4% ammonium succinate. Of seven vitamins tested, a deficiency for thiamine alone was demonstrated.

Introduction

Atropellis piniphila (Weir) Lohman and Cash is an inoperculate discomycete associated with a canker which causes considerable damage to lodgepole pine in several areas of Alberta, British Columbia, and the western United States. The fungus was briefly described by Weir (1921) under the name *Cenangium piniphilum* n. sp. He reported growth of isolates in culture, but provided no information on their characteristics in culture or on the media employed. Lohman and Cash (1940) revised the genus *Atropellis* and incorporated *C. piniphilum* in this genus. The present studies were carried out as part of an investigation into the canker disease of lodgepole pine. The capacity of various media to support growth of this fungus was studied and cultures on malt agar described. Studies were undertaken to determine the influence of temperature, pH, carbon–nitrogen concentrations, and vitamin deficiencies on growth of the fungus.

Culture Description

MATERIAL AND METHODS

The 24 isolates employed for the culture descriptions were collected from six locations in British Columbia, four in Alberta, and one in Idaho. Four of the isolates were obtained from wood; the remaining 20 originated from single ascospores. Difco materials were used to prepare the media, with the exceptions of soya-digest–dextrose and oat-digest–dextrose agars which were prepared from Ben Venue products. Twenty milliliters of agar was added to each Petri dish. Cultures used for comparative growth measurements were inoculated with a disk of mycelium, approximately 1.5 mm in diameter, cut from the growing edge of a colony. The disks were cut with thin-walled glass capillary tubing mounted in a holder. All cultures were incubated at

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16±1.5° C. The relative humidity of cabinets used to grow cultures for conidial descriptions was approximately 50%; all other cultures were grown at approximately 10% relative humidity. Unless otherwise stated, culture descriptions were prepared from 6-weeks-old colonies on malt agar. Color descriptions are provided in both the Munsell numerical notation (1942) and in the equivalent names given by the U.S. National Bureau of Standards (1955). Other descriptive terms are largely those adopted by Nobles (1948). Estimates of comparative growth rates on diverse media were obtained by combining data on colony diameters with subjective estimates of the depth and density of the colonies.

RESULTS

Growth Rate

Growth was slow on all media tested. It was most rapid on soya-digest–dextrose, oat-digest–dextrose, and Sabouraud's agar; slightly slower on potato–dextrose agar and on V8-juice agar with an initial pH of 4.5 (Miller 1955); and considerably slower on malt and yeast-extract agars. No growth occurred on Czapek–Dox or trypticase–soya agars. The mean colony diameters of all the isolates at weekly intervals on malt agar are given in Table I. Considerable variation occurred between isolates, particularly in older cultures. This was apparently caused partly by differences in over-all growth rates and partly by differences in the shapes of colonies.

TABLE I

Growth of *Atropellis piniphila* on malt agar at 16° C

Growth period, weeks	Mean colony diameter, mm
1	5.1±0.16*
2	7.9±0.29
3	11.2±0.34
4	14.7±0.45

*Standard error.

Growth Characters (Figs. 1–6)

Most colonies possess a central mound 2–3 mm above the level of the appressed margin. However, four isolates possess an entirely appressed form, while two others form 'hedgehog'-shaped colonies of small diameter. The texture is velvety or chamoislike, except for one isolate which is crustose. The margin is usually even and appressed, but it may become bayed in older cultures. The cultures with a 'hedgehog' form possess virtually no projecting margin.

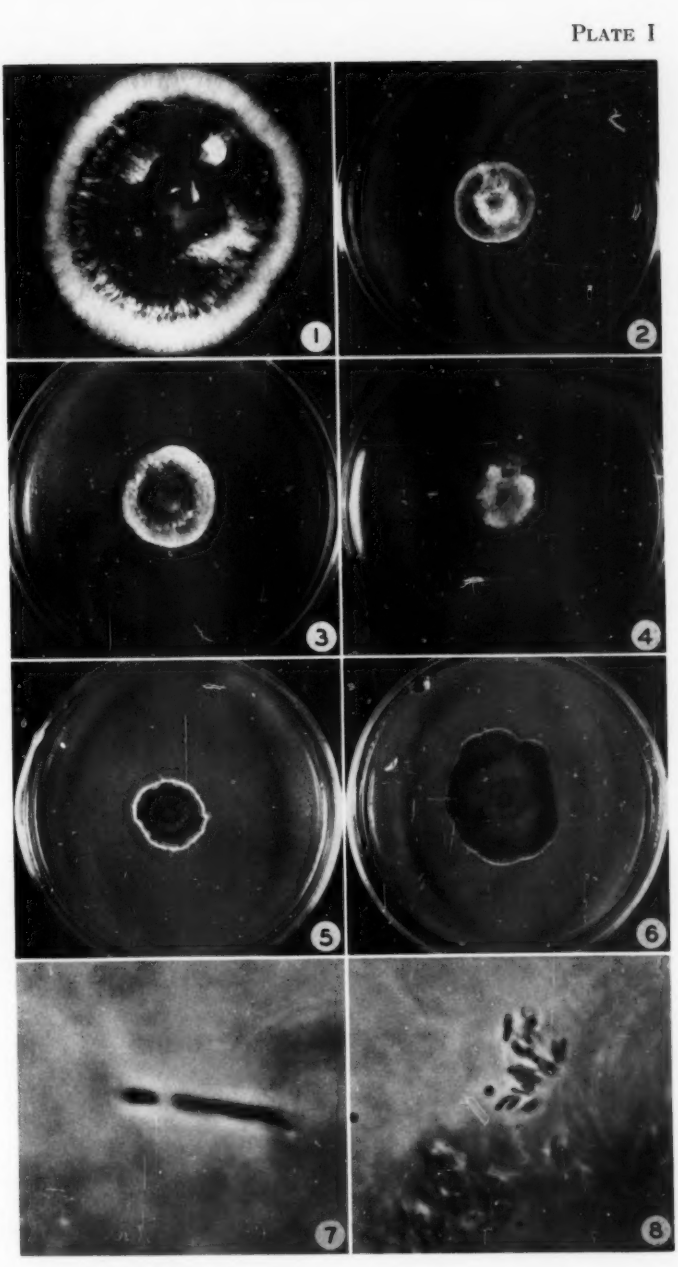
The color of the upper surface varies considerably between isolates and six groups can be distinguished. However, the reverse surface is very similar

FIGS. 1–6. Six-weeks-old cultures of *Atropellis piniphila* incubated on malt agar at approximately 50% relative humidity and 16° C: 1, under side of isolate N 58; 2, isolate N 47; 3, isolate N 50; 4, isolate N 57; 5, isolate N 54 with drops of exudate containing spores; 6, isolate N 53 with several drops of exudate.

FIG. 7. Conidiophore of *Atropellis piniphila* (×2550), under phase contrast, showing endogenous spore formation.

FIG. 8. Group of conidia (×830) under phase contrast.

PLATE I



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throughout these groups; it consists of a broad central zone of dark grayish yellowish brown (10.0 YR 2–4/2.0) with a narrow translucent margin of yellowish gray (10.0 YR 8.0/2.0). The upper surface in all the isolates is opaque, except for a translucent margin. Eight isolates possess a yellowish gray center (10.0 YR 5–8.0/2.0) with a flecking of grayish purple (7.5 RP 5–6.0/2.0) and a growing margin of pale purplish pink (7.5 RP 8.5/2.0). Another group of five isolates possess a central zone of dark grayish purple (7.5 RP 2.5–4.0/2–3.0), with a flecking and margin of light purplish gray (7.5 RP 7–8.0/2.0), with the exception of one isolate with a margin of light olive brown (1.0 Y 5.0/2.0). Four other isolates exhibit a small central zone of light grayish yellowish brown (10.0 YR 7.0/3.0) surrounded by a broad yellowish gray zone (10.0 YR 8.0/2.0) and a margin similar to the one last described. A further three isolates possess a grayish brown central zone (5.0 YR 3.0/2.0) with flecks of light brown (5.0 YR 6.5/4.0) and gray (N 8.5), and with a growing margin of yellowish gray (10.0 YR 8.0/2.0). The light brown color present as flecking in the rest of the group predominates in the central zone of one isolate. One other isolate is dark grayish yellowish brown (10.0 YR 2–4.0/2.0) over the entire surface. Three other isolates exhibit a gray color over the central area (N 7.5–8.5).

Growth at 50% relative humidity produces colonies fairly similar to those grown at the lower humidity, that is, at 10%. At the higher humidity the colonies tend to be denser and deeper, and the color balance is occasionally affected; a dark grayish purple color is produced by a larger number of isolates at the higher humidity. At 50% relative humidity, most isolates produce droplets of viscous liquid at their centers when grown on malt agar; few produce them on malt at the lower humidity. However, even at the lower humidity, many isolates produce these droplets when grown on oat-digest–dextrose or soya-digest–dextrose agars. The color of the droplets also varies according to the humidity during growth. Colonies grown at the higher level possess yellowish gray (2.5 Y 8.0/2.0) droplets owing to the presence of numerous conidia within them. At the lower humidity, the droplets are dark grayish brown (5.0 YR 2.0/2.0) and contain no conidia.

In the liquid synthetic medium employed for the nutritional studies, the fungal pigments exhibit indicator properties. The mycelium is hyaline at pH 5.5, with a progressively darker gray produced with lower values down to 4.2; below 4.2 a reddish hue appears which intensifies until at 2.8 a grayish red (10.0 R 5.0/2.5) results. Pigmentation is also affected by the position of the mycelium in liquid media; hyphae on the surface are much more intensely pigmented than those beneath the surface.

Hyphal Characters

Hyphae in the advancing zone of 6-weeks-old colonies are fairly similar in all isolates. The hyphae are hyaline, thin-walled, infrequently branched, of uniform shape, 1.0–3.5 μ in diameter, with cells 12–52 μ long. The cells are densely protoplasmic and stain intensely with trypan blue in lactophenol.

Aerial hyphae of all isolates are of two types which differ in width, pigmentation, and other characteristics. One type is hyaline, relatively straight, and infrequently branched, with short and thin-walled cells 0.5–1.8 μ in dia-

meter and 4–7 μ long which stain intensely in trypan blue. Hyphae of this type are fairly similar in all isolates. The second hyphal type occurring in the aerial position exhibits greater variation between isolates. This type is strongly pigmented, thick-walled, frequently branched, nonstaining in trypan blue, and possesses numerous cells of irregular shape. Regularly shaped hyphae are 2.2–5.5 μ in diameter and 5.0–9.0 μ long. The characteristics of this type which vary most between isolates are: frequency of septation, regularity of cell shape, occurrence of vesicles, and possession of verrucose walls. Three isolates form little pigment in the aerial hyphae.

Submerged hyphae resemble the narrow hyaline type described above, except that they are frequently branched and usually sinuous. Their cells are normally 1.0–3.0 μ in diameter, occasionally up to 18 μ. They have dense cellular contents that stain readily in trypan blue. Hyphae close to the surface are often slightly pigmented and produce a browning of the surrounding agar.

The hyphae of colonies grown at 50% relative humidity are fairly similar to those described, but they tend to produce fewer vesicles and thinner walls with less-frequent verrucose projections.

Chlamydospores are produced in shake culture on the necks of flasks just above the uppermost level of the media. They are thick-walled, dark-colored, varying from 7 to 12 μ in diameter, or occasionally larger, and are formed in the intercalary and terminal positions.

Several unsuccessful attempts were made to induce apothecial formation in culture. These involved the use of treatments applied to 6-weeks-old cultures grown from multispore inoculum. Treatments included ultraviolet irradiation of cultures grown on a variety of media; transfer of cultures to high and low temperatures; growth in constant fluorescent light of various intensities; growth in alternating light and dark; transfer to media of low nutrient concentration; and growth in media containing several concentrations of all seven vitamins.

Phialide-type conidiophores (Fig. 7) are produced on 6-weeks-old colonies grown at a relative humidity of approximately 50% or higher. They occur in groups scattered through the aerial and upper submerged hyphae of the central zones; a few isolates produce them to within several millimeters of the growing edge of the colony. They are thin-walled, hyaline, 0.8–1.3 by 14–25 μ, with an open distal end which is usually empty in slide mounts.

Conidia (Fig. 8) are thin-walled, hyaline, cylindrical, 0.5–0.9×4–6 μ, and stain readily with trypan blue in lactophenol. Some have a small hyaline papilla at one end. The conidia are produced endogenously in basipetal succession from the proximal end of the conidiophores, and may become slightly allantoid after release. Each conidium develops as an abstriction of the protoplasm which remains attached by a fine cytoplasmic connection until the spore reaches full size. The conidia are extruded from the conidiophores and collect in groups within the colony or in drops of exudate.

Physiology

MATERIAL AND METHODS

Unless otherwise stated, the medium employed, similar to the one used by Taber and Vining (1957), contained the following constituents: glucose, 10 g;

ammonium succinate, 4 g; mineral concentrate, 100 ml; each vitamin solution, 20 ml; distilled water to 1 liter.

The mineral concentrate consisted of: K_2HPO_4, 500 mg; KH_2PO_4, 3.5 g; $MgSO_4.7H_2O$, 1.005 g; $CaCl_2.2H_2O$, 29.75 mg; $FeSO_4.7H_2O$, 25 mg; $ZnSO_4.7H_2O$, 22 mg; $MnSO_4$, 13.75 mg; NaCl, 12.75 mg; $CuCl_2.2H_2O$, 1.34 mg; $(NH_4)_6Mo_7O_{24}.4H_2O$, 9.10 mg; distilled water to 500 ml.

The vitamins were prepared separately so that 20 ml of each vitamin solution contained one of the following constituents: biotin, 5 μg; thiamine–HCl, 100 μg; *i*-inositol, 1 mg; pyridoxine–HCl, 100 μg; Ca-pantothenate, 100 μg; nicotinamide, 50 μg; *p*-aminobenzoic acid, 50 μg.

The constituents were grouped into three sets, each of which was sterilized separately, and then combined when cool. The glucose was prepared separately and sterilized by autoclaving. The ammonium succinate was combined with the mineral solution and the pH adjusted to 5.0 before autoclaving. Vitamins were sterilized by filtration through fritted glass filters of ultrafine porosity. Flasks containing solutions to be autoclaved were covered with aluminum foil. The requisite volume of sterile water was added to the combined constituents before dispensing 50 ml of the complete medium to each Erlenmeyer flask. Glassware was thoroughly cleaned by immersion first in detergent, then in chromic acid, and finally in several rinses of distilled water. A Beckman meter was used for pH measurements.

The inoculum for all the experiments, except for vitamin studies, was grown on oat-digest–dextrose agar in 1-in.-diameter tubes, for 3 weeks at 14° C. The entire colony from each tube was then homogenized in a sterile Waring blendor for 1 minute and the resulting suspension made up to 60 ml with sterile distilled water. Each flask of medium was inoculated with 0.6 ml of the suspension. Incubation was carried out for 5 to 7 weeks in still culture at 14±1° C. The mycelium was harvested by filtration on individually tared filter papers, washed with distilled water, and dried for 48 hours at 80° C. Before it was weighed, the mycelium was left for 4 hours in a desiccator at room temperature. Growth was recorded as the mean of three to four replicates.

RESULTS

Temperature Relations

The effect of temperature on the growth of isolate N 19 was tested in a medium containing biotin, thiamine, and inositol (Table II). The flasks were incubated at 4°, 10°, 14°, 18°, 24°, and 28° C for 50 days, with occasional agitation. Growth was barely detectable at 4° C, reached a maximum at 18° C, and ceased at 28° C.

TABLE II

The effect of temperature on growth of isolate N 19

Temperature, °C	Mean dry weight mycelium, mg
4	1.3±0.6*
10	57.8±3.23
14	65.7±2.55
18	82.8±7.6
24	18.8±2.0
28	0.0

*Standard error.

pH Relations

The effect of pH of the culture medium on growth of isolate N 19 was studied. The standard medium, containing biotin, thiamine, and inositol, and modified by the addition of McIlvaine's buffer (Handbook of Chemistry and Physics, 1953), was prepared in several sets corresponding to the number of pH values. The appropriate buffer mixture was added to each set of media after adjusting the pH of each set by adding solutions of hydrochloric acid or sodium hydroxide. The quantity of buffer added was adjusted so that its concentration in the final medium was equal to that obtained by mixing the appropriate volumes of 0.1 *M* disodium phosphate with 0.05 *M* citric acid for the pH required. The volume of sterile distilled water was adjusted so as to maintain the standard concentration of each constituent. After inoculation the flasks were incubated for 55 days, with occasional agitation.

The results are presented in Table III. The pH stability indicates that sufficient buffer was added. The results show an optimum range from 3.0 to 4.0. A considerable reduction in growth occurred at 0.5 unit above and below this range. However, the growth rate was still fairly rapid at 2.5 and 4.5, but it fell to a very low value at 5.0 and increased only slightly at 5.5.

TABLE III

The effect of pH on growth of isolate N 19 in a buffered medium

Initial pH	Final pH	Mean dry weight mycelium, mg
2.5	2.7	28.9±3.1*
3.0	3.1	40.8±1.9
3.5	3.5	43.2±5.1
4.0	4.0	46.3±2.5
4.5	4.5	24.1±1.8
5.0	5.1	3.3±0.8
5.5	5.6	8.9±0.5

*Standard error.

Carbon–Nitrogen Balance

The influence on growth of different proportions and concentrations of carbon and nitrogen was studied by comparing the weights of mycelia produced in several sets of media which differed only in the concentrations of dextrose and ammonium succinate present. Four concentrations of dextrose (0.5% (w/v), 1.0%, 2.0%, and 4.0%) and two concentrations of ammonium succinate (0.4% and 0.8%) were used. The vitamins biotin, thiamine, and inositol were supplied. Cultures of isolate N 19 were incubated for 45 days with occasional agitation. The results are shown in Table IV.

The maximum weight of mycelium was produced in media containing a combination of 4.0% dextrose and 0.4% ammonium succinate. Considerable growth occurred in all media containing 0.4% ammonium succinate, with a slight increase produced by raising the dextrose concentration from 0.5% to 1.0%; further increases of dextrose produced very little additional growth. Growth was less in media containing 0.8% ammonium succinate than in the equivalent set containing only 0.4%.

TABLE IV

The effect of the carbon–nitrogen balance on the growth of the isolate N 19

% (w/v) dextrose	% (w/v) ammonium succinate	Mean dry weight mycelium, mg
0.5	0.4	78.5± 2.5*
1.0	0.4	95.8± 5.3
2.0	0.4	94.6±12.3
4.0	0.4	103.0± 3.7
0.5	0.8	57.9± 3.0
1.0	0.8	54.9± 3.5
2.0	0.8	56.8± 2.4
4.0	0.8	74.7± 3.4

*Standard error.

Vitamin Requirements

Information on vitamin requirements was obtained by comparing the weights of mycelium produced in media from which one vitamin was omitted with that produced in media containing all, or none, of the vitamins concerned. The study was carried out in three experiments. The first two dealt with the separate influence of biotin, thiamine, inositol, pantothenate, pyridoxine, nicotinamide, and *p*-aminobenzoic acid. The third experiment dealt with several concentrations of biotin and thiamine which the earlier work had indicated should receive further investigation.

Except for the treatment of the vitamins and a number of extra precautions associated with their study, the media were prepared in the standard way. Care was taken to minimize the transfer of nutrients in the inoculum by growing it in agar media which had been purified by leaching with hot distilled water followed by ethanol. The inoculum, after homogenization, was washed by centrifuging and resuspending it four times in physiological saline, and finally in distilled water. The inoculum was used within 24 hours of preparation. Following the standard cleaning, the glassware was again thoroughly rinsed in distilled water. In the first two experiments, flasks containing media constituents were covered with aluminum foil during autoclaving, but this was replaced with fresh cotton plugs when cool. In the experiment involving only biotin and thiamine, the flasks were covered with uncoated cellophane throughout the preparation and incubation as a special precaution against vitamin contamination (Jennison *et al.* 1955). The incubation period for the first experiment involving biotin, thiamine, and inositol was 58 days; that for the second experiment involving pantothenate, pyridoxine, *p*-aminobenzoic acid, and nicotinamide was 46 days; and the period for the third experiment was 49 days. The first two experiments, the results of which are presented in Tables V and VI respectively, utilized both isolates N 19 and N 21, while the media in the third experiment were inoculated with N 21 alone. All seven vitamins were incorporated in the controls of the first two experiments.

The thiamine-deficient medium produced considerably less mycelium than the media containing all the vitamins, but much more mycelium than the media containing no vitamins. This difference might have been caused by contamination of thiamine from an unknown source. The third experiment

utilizing biotin and thiamine alone was carried out to test these possibilities. Biotin was included because of the minute concentration required by most fungi with this deficiency and the consequent danger of contamination. In this experiment, thiamine was employed in three concentrations of 100, 200, and 400 μg/l., and biotin in 5, 10, and 20 μg/l. Two control series were used, one with no vitamins, and the other containing biotin and thiamine at the lowest concentration indicated above. The results, presented in Table VII, indicate a total deficiency for thiamine, with very little increased mycelium produced by increasing the concentration from 100 to 400 μg/l. This suggests that the earlier result in which some mycelium was produced in the apparent absence of thiamine was due to the accidental introduction of the latter from an unknown source, possibly one of the other vitamins. The results of all three experiments indicate that the isolates were able to synthesize sufficient vitamins, other than thiamine.

TABLE V

The effect of biotin, thiamine, and inositol on growth of isolates N 19 and N 21

Vitamin omitted	N 19, mean dry weight mycelium, mg	N 21, mean dry weight mycelium, mg
None	89.6±5.9*	67.8±3.4
All	2.1±0.5	1.8±0.6
Biotin	78.5±2.1	69.2±4.0
i-Inositol	84.0±3.2	73.4±6.0
Thiamine	31.5±4.2	30.7±5.7

*Standard error.

TABLE VI

The effect of pantothenate, pyridoxine, nicotinamide, and *p*-aminobenzoic acid on growth of isolates N 19 and N 21

Vitamin omitted	N 19, mean dry weight mycelium, mg	N 21, mean dry weight mycelium, mg
None	59.1±4.8*	63.3±0.8
All	2.2±0.6	3.7±0.5
Calcium pantothenate	61.0±3.8	71.6±4.6
Pyridoxine	53.0±3.9	70.4±2.4
p-Aminobenzoic acid	57.2±3.9	66.4±1.6
Nicotinamide	62.8±5.6	63.4±6.2

*Standard error.

TABLE VII

The effect of different concentrations of biotin and thiamine on growth of isolate N 21

Vitamin added	Vitamin conc., μg/l.	Mean dry weight mycelium, mg
Biotin and thiamine	5 100	65.9±1.7*
None		—
Thiamine	100	56.0±1.6
Thiamine	200	57.3±3.8
Thiamine	400	63.4±2.8
Biotin	5	4.5±1.7
Biotin	10	2.7±1.0
Biotin	20	4.5±1.4

*Standard error.

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PRELIMINARY STUDIES ON THE UTILIZATION AND RELEASE OF AMINO ACIDS BY CYLINDROCARPON RADICICOLA WOLL.[1]

R. G. ATKINSON[2]

Abstract

One-dimensional paper chromatographic studies demonstrated that, in the absence of inorganic nitrogen, shake cultures of *Cylindrocarpon radicicola* Woll. preferentially utilized glutamic acid, proline, phenylalanine, and leucine from a basal salts – dextrose medium containing also histidine, aspartic acid, threonine, and valine. A similar analysis of slower-growing stationary cultures in the same medium failed to reveal this pattern of amino acid uptake. The growth of *C. radicicola* in a basal salts – dextrose medium containing KNO_3 as the sole source of nitrogen resulted in the appearance of numerous amino acids in both stationary and shake culture fluids. The release of amino acids in stationary culture increased from a low to a high level after a growth period of 7 and 14 days, respectively. In shake culture, however, the opposite trend occurred.

Introduction

With a few exceptions, fungi, other than induced mutant strains, do not require amino acids *per se* for growth. The only exceptions to this were noted by Cochrane (3). Nevertheless, many species grow better when amino nitrogen is present than when only inorganic nitrogen is available. Certain amino acids promote greater fungal growth than others, indicating that the availability of the amino group for nitrogen metabolism varies considerably. Does it follow then that fungi growing in the presence of a mixture of amino acids will utilize certain ones preferentially? Pyle (13) found no selective absorption by *Penicillium chrysogenum* Thom of the 11 amino acids detected chromatographically in corn steep liquor medium. In contrast, Proom and Woiwod (12) found that bacteria were highly selective in their utilization of amino nitrogen when grown in a mixture of amino acids.

On the other hand, fungi may release amino acids into culture fluids, as has been shown by De Vay (4), McAnelly (7), Mackenzie and Cook (6), Morton and Broadbent (8), Murray and Zacheile (9), Rao and Venkataraman (14), and Reindel and Hoppe (15). Pfennig (10) studied the release of amino acids into culture fluids by *Streptomyces* sp.

Cylindrocarpon radicicola Woll. is a widespread, soil-borne fungus and has been implicated in root rots of raspberry (1) and strawberry (2, 16) in which a complex of soil microorganisms is held to be responsible. The present work was initiated as part of a study of the physiology of certain root rot fungi to complement investigations on their parasitic behavior. The data presented here represent the initial experiments of a series it was not possible to continue. They are considered to be of sufficient interest, however, to publish at this time in the hope of stimulating more-detailed studies on the amino acid metabolism

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of plant pathogenic fungi. This preliminary investigation was designed to study the utilization by *C. radicicola* of individual members of a known mixture of amino acids and, conversely, the release of amino acids by this fungus in the absence of organic nitrogen in the medium.

Materials and Methods

Cultures

The test fungus selected, *Cylindrocarpon radicicola* Woll., was originally isolated from strawberry roots suffering from the "root rot" condition found in the Niagara Peninsula of Ontario. The fungus was grown in two kinds of liquid media differing only in their source of nitrogen as follows:

Nitrate medium.—KH_2PO_4, 1.0 g; $MgSO_4$, 0.5 g; $CaCl_2$, 0.5 g; $FeCl_3$, 0.02 g; KNO_3, 3.0 g; $ZnSO_4$, 0.02 g; $MnCl_2$, 0.02 g; dextrose, 10.0 g; distilled water, 1000 ml; reaction adjusted to pH 6.6; nitrogen supplied = 415.6 mg/liter.

Amino acid medium.—As in the nitrate medium with KNO_3 replaced by the following mixture of amino acids, each in a concentration of 3 μM: *l*-histidine, *l*-aspartic acid, *l*-glutamic acid, *dl*-threonine, *dl*-proline, *dl*-valine, *dl*-phenylalanine, *l*-leucine; reaction adjusted to pH 6.6; nitrogen supplied = 420.2 mg/liter.

All glassware was cleaned in a sulphuric acid–potassium dichromate cleaning solution, then well rinsed in tap water with a final rinse in distilled water.

Both media were prepared in 50-ml amounts in 250-ml Erlenmeyer flasks and sterilized at 15 lb steam pressure for 20 minutes.

The amino acid medium was prepared by first dissolving the amino acids in the nitrate medium with the KNO_3 omitted. After filter-sterilizing by means of an ultrafine sintered glass filter 10-ml aliquots were added aseptically to 40-ml amounts of steam-sterilized nitrate medium, also lacking the KNO_3.

A very dilute mycelial suspension of *C. radicicola* was prepared in sterile distilled water by picking small tufts of aerial hyphae from a potato–dextrose–agar slant culture, carefully avoiding the agar surface to prevent the introduction of extraneous amino acids into the test media. A 1-mm loopful of the mycelial suspension was used to inoculate the media so that only one or two minute pieces of inoculum were introduced to each flask.

Inoculated flasks of both media were incubated at the same room temperature under stationary[3] and shake culture conditions. Uninoculated flasks of the amino acid medium served as controls.

Chromatographic Analysis

After 7 and 14 days, two stationary and two shake cultures in both media, plus control flasks, were removed at each time to a refrigerator to await processing.

Cell-free culture fluids were prepared for amino acid analysis by centrifuging the media and then filtering the supernatant liquid through a sintered glass filter. The mycelium was washed in several changes of distilled water and dried at 70° C before being weighed.

The cell-free culture fluid was evaporated in a rotating evaporating flask under vacuum in a water bath at 37° to 39° C. The moist residue was taken up

[3]Only one large colony developed in each of the stationary cultures in both media.

in 10 ml of 80% ethyl alcohol and centrifuged to remove the undissolved components of the mixture. The supernatant was carefully removed and desalted following the method of Plaisted (11). Solutions with initial pigmentation were clear after passage through the resin, the colored component being adsorbed on approximately the uppermost centimeter of the column.

The desalted eluate from the resin column was evaporated at 37° to 39° C as described above and the residue taken up in 1 ml of 10% isopropyl alcohol. Suitable portions of this final concentrated solution from the culture fluid were analyzed for amino acid content by one-dimensional descending paper chromatography using No. 2 Whatman Chromatographic Paper.

The solvent employed was *n*-butanol: acetic acid: water in the proportion of 4:1:5. The indicator spray used was that of Kolor and Roberts (5). Proline reacts with this indicator to produce a diagnostic brilliant pink-red color in sharp contrast to the various shades of purple developed in the presence of other amino acids.

Results and Discussion

Amino Acid Cultures

The growth of *C. radicicola* for 7 days in stationary culture did not result in a detectable reduction in concentration of any of the eight amino acids originally present in the amino acid medium. In fact, two extraneous amino acids, one in trace amounts, now appeared in the culture fluid. Even after 14 days, only relatively small reductions in the amino acid content were detected, as shown in Table I. This slight depletion was noticeable with aspartic acid, glutamic acid, threonine, and proline. The two extraneous amino acids noted above after 7 days were again present after 14 days but in greater amounts.

TABLE I

The pattern of utilization of eight amino acids in liquid culture by *Cylindrocarpon radicicola* Woll. as determined by one-dimensional paper chromatography

Amino acids in order of increasing R_f values	Relative size and color intensity of ninhydrin reaction			
	Stationary culture		Shake culture	
	7 days	14 days	7 days	14 days
1. Histidine	+++++*	+++++	+++	0
2. Aspartic acid	+++++	++++	+++	Tr.
3. Glutamic acid	+++++	++++	+	0
4. Threonine	+++++	++++	+++	Tr.
Alanine (?) †	+	++	+++	Tr.
5. Proline	+++++	++++	++	0
Tyrosine (?) †	Tr.	++	++	0
6. Valine	+++++	+++++	++++	0
7. Phenylalanine	+++++	+++++	++	0
8. Leucine	+++++	+++++	++	0

*+++++ = Maximum size and color intensity of amino acid – ninhydrin reaction, decreasing in order of decreasing number of 'plus' signs.
Tr. = trace only of ninhydrin reaction.
0 — Denotes absence of ninhydrin reaction.
†Ninhydrin-positive areas not present in chromatograms of the uninoculated control medium.
(?) — Provisional identification only, with reference to the chromatogram of the standards of the 14 amino acids run on the same sheet.

On the other hand, growth in shake cultures for 7 days resulted in a definite reduction in the concentration of all eight amino acids that was especially marked with glutamic acid, proline, phenylalanine, and leucine, originally supplied in the medium. In fact, glutamic acid was almost completely utilized. Two extraneous amino acids with R_f values similar to those noted above in the stationary culture fluids were also present in the shake culture fluids, but in greater amounts. After 14 days under shake culture conditions only a trace of three amino acids remained in the amino acid culture fluids. The more rapid rate of depletion of amino acids in the shake culture fluids, resulting in their almost complete absorption after 14 days of growth, contrasts sharply with the very slow reduction of the amino acid content in the stationary culture fluids, and was positively correlated with the weight of mycelium produced, as shown in Table II.

TABLE II

The effect of nitrate, and of amino, nitrogen on the mycelial weights of *Cylindrocarpon radicicola* Woll. growing in stationary and in shake cultures

Source of nitrogen	Days of growth	Weight of mycelium (mg)							
		Stationary culture				Shake culture			
		Rep. 1	Rep. 2	Rep. 3	Av.	Rep. 1	Rep. 2	Rep. 3	Av.
KNO_3	7	31.0	23.5	—	27.3	113.0	70.5	—	91.8
	14	67.0	93.0	97.5	85.8	181.0	177.5	195.0	184.5
Amino acids	7	44.0	47.5	—	45.8	207.0	161.5	—	184.3
	14	186.0	192.0	145.3	174.4	233.0	254.0	259.0	248.6

Nitrate Cultures

When *C. radicicola* was grown in the presence of basal salts, dextrose, and KNO_3 as the sole source of nitrogen a great variety and quantity of amino acids appeared in the culture fluids under both stationary and shake conditions.

After a growth period of 7 days under stationary conditions, 9 amino acids were found in the culture fluid. These same 9 amino acids were also present after 14 days but, with one exception, in much greater quantities than at 7 days. Three additional amino acids were also detected after 14 days, making a total of 12 at this time.

In the 7-day-old shake cultures 11 amino acids were detected in the nitrate medium, of which nine had R_f values similar to those in the stationary cultures mentioned above. After 14 days, however, one less amino acid was detected and the concentration of the other nine had, for the most part, declined considerably below the level at 7 days. Table III presents the relative concentrations of the amino acids released into the nitrate medium as affected by stationary and shake culture conditions.

In summing up then, these studies have pointed up some interesting aspects of the nitrogen metabolism of *C. radicicola*, which has been shown to absorb amino acids with some degree of preference in the absence of nitrate, but which readily released amino acids when grown in the presence of nitrate as the sole source of nitrogen.

TABLE III

The effect of stationary and shake culture conditions on the release of amino acids by *Cylindrocarpon radicicola* Woll. into a basal nitrate liquid medium

Amino acids in order of increasing R_f values	Stationary culture		Shake culture	
	7 days	14 days	7 days	14 days
1.*	Tr.	++	++	++
2.	0	+	++	++
3.	0	+	+++	+
4.	++	++++	+++++	+++
5.	++	+++++‡	+++++	+++
6.	++	+++++	+++++	+++
7.	+++	+++++	++	+
8. Proline†	Tr.	+++	+++	Tr.
9.	+	++++	+++	+
10.	+	+++	Tr.	0
11.	Tr.	Tr.	++++	+
12.	+	++++		

*Point of origin of the chromatogram.
†Proline was readily identified on the chromatogram by its diagnostic reddish color reaction with the indicator spray formulation employed.
‡++++++ = Maximum size and color intensity of amino acid – ninhydrin reaction.

As might be expected, the much greater amount of mycelium produced in shake than in stationary culture was correlated with the much more rapid utilization of amino acids in the former instance. More puzzling, however, was the release of large quantities of amino acids in the presence of nitrate as the sole source of nitrogen, which accumulated with time in stationary cultures, but which declined over the 14-day test period in shake cultures. It is interesting to note that, although large quantities of the amino acids released from the mycelium into the nitrate medium remained at the end of the 14-day experimental period, the amino acids originally supplied in the second medium had been almost completely utilized under shake conditions, when nitrate nitrogen was absent.

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NUCLEAR AND CYTOPLASMIC FACTORS IN DEVELOPMENT OF CHLOROPLAST STRUCTURE AND FUNCTION[1]

DITER VON WETTSTEIN

The formation of chloroplast structure is controlled by the genome, the plastome, and the plasmone (Table I). Alteration, destruction, or inactivation of the hereditary factors concerned with plastid development lead to a great variety of chloroplast defects, which have been found in every species of higher and lower plants studied closely. The way in which these defects are inherited can tell us whether they are caused by chromosomal or extranuclear mutations, the latter showing a non-Mendelian transmission to the offspring (cf. 5). Gene-mutations and deficiencies affecting the chloroplast can be produced in unlimited number by ionizing radiations and chemical mutagens. In most cases they behave as simple recessives.

TABLE I

Genetic systems controlling chloroplast structure and function

	Localization	Studied with:	Origin	Example
Genome	Chromosomes	Gene-mutations	Spontan.	Barley
		Deficiencies	Induced	Corn
Plastome	Extranuclear	Mutations	Spontan.	*Nicotiana*
		Hybrids		*Oenothera*
		Gene-dependent plastome-mutations	Spontan.	Iojap in corn
			Induced	Barley
Plasmone	Extranuclear	Variegated form	Spontan.	*Humulus*

Among the extranuclear mutations several different types can be distinguished. Certain *albomaculatus* types show a distinct somatic segregation, the offspring from green, white, or yellow and from variegated flowers being different. They behave as if the variegated plants contain two or more different sorts of plastids, which during the ontogeny of the plant seem to be sorted out. Such chloroplast differences are said to be conditioned by the plastome, implying that plastids have far-reaching genetic autonomy (16, 17, 18). At present no conclusive proof exists that the genetic factors causing these plastid defects are really located in the plastids themselves (34), but for the time being we may use the term plastome to designate the sum of the extranuclear factors affecting chloroplasts that show the type of inheritance just described. Plastome differences can be studied in *Oenothera* hybrids, where certain genomes do not harmonize with certain plastomes, or in plastome mutants

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producing the defect under all tested genome combinations. In addition there are the gene-dependent or gene-induced plastome mutations (19, 20, 1). Here a recessive gene causes a plastid defect and this defect is subsequently inherited according to the status *albomaculatus*, regardless of whether the recessive gene or its wild type allele is present. This type of plastome mutation can be induced by ionizing radiations (39).

The so-called plasmone mutations also show a variegated phenotype but here no somatic segregation of the factors causing the plastid defect is observed. The offspring of green, white, and variegated flowers when used as female parent is uniform and variegated. This type of variegation occurs in *Humulus japonicus* (cf. 5).

For a number of years we have tried to analyze the action of these different genetic systems on chloroplast structure and development. Relatively rapid progress has been made in understanding the action of genes on chloroplasts. The lethal gene mutations block a step in one of the synthetic pathways necessary for the differentiation or function of the chloroplast. This is reflected by a block in the development of the macromolecular structures of the chloroplasts, which in suitable mutants can be characterized by electron microscopic analysis. The causal analysis of chlorophyll defects showing extranuclear inheritance is less advanced. At present we can describe the phenotype of different plastome and plasmone mutants in terms of the submicroscopic structure of their plastids and mitochondria.

Gene-Mutation and Chloroplast Development

The mature chloroplast of angiosperms contains a lamellar system embedded in the granular stroma (cf. 6, 9). The lamellar system consists of disks (Fig. 3), which in the grana regions are tightly aggregated. As can be seen from the tracing of this micrograph (Fig. 2) about every other disk in the grana package continues into the stroma, and into an adjacent granum. The disk membranes are about 30 to 40 Å thick and enclose an electron-optically relatively empty space. Between the disks in the grana regions lies a thin layer of material (ca. 20 Å thick), which scatters electrons more than the material inside the disk.

The essential features of the development of a chloroplast are given in Fig. 1 (34, 36). In the small precursors of plastids (proplastids) the inner of the two membranes which separate the organelles from the cytoplasm invaginates and produces a number of vesicles (Fig. 4). In this stage a clear-cut distinction between proplastids and mitochondria is not possible at present. As more vesicles accumulate, they align into primary layers. The vesicles seem to fuse and form larger disks, and we find in more advanced stages packages of aggregated disks having the same structure as the grana in the mature chloroplast. Further differentiation consists of an increase in the number of the disks, so that the grana piles become higher. Eventually the grana are connected by extended disks through the stroma. Both the arrangement of all the vesicles into primary layers and the aggregation of the disks into grana require light.

As indicated in Fig. 1, in darkness or under insufficient light conditions the vesicles accumulate to form a prolamellar body. In darkness, grana formation,

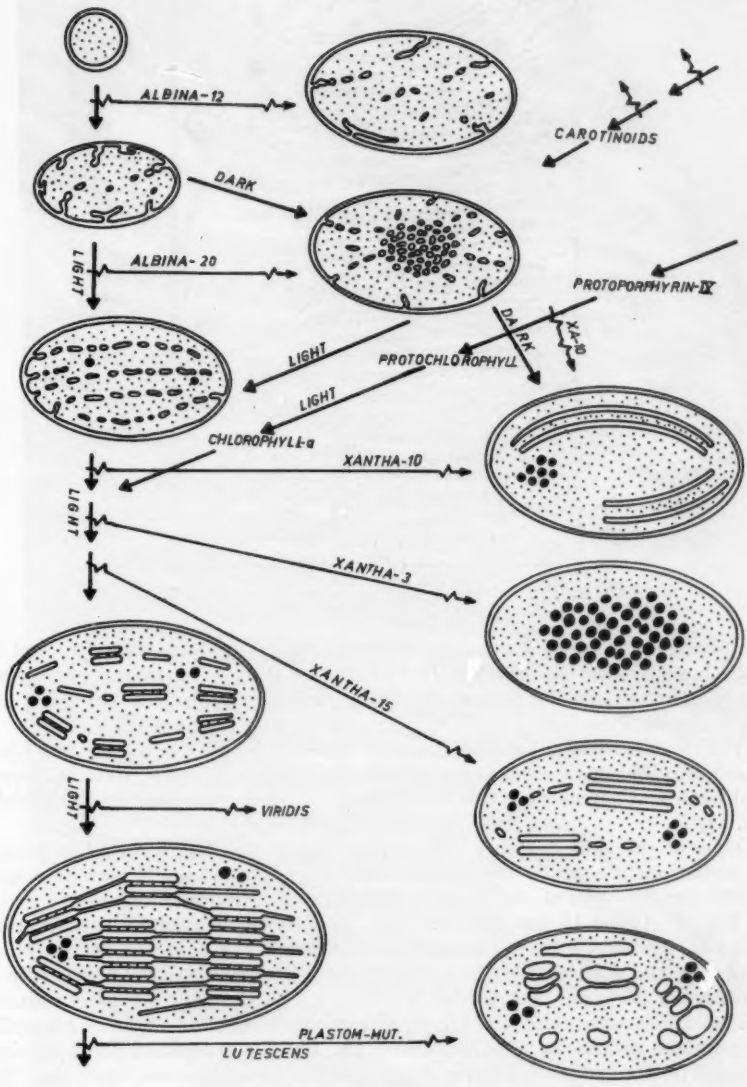

FIG. 1. Diagram on the development of plastids in normal plants and various mutants.

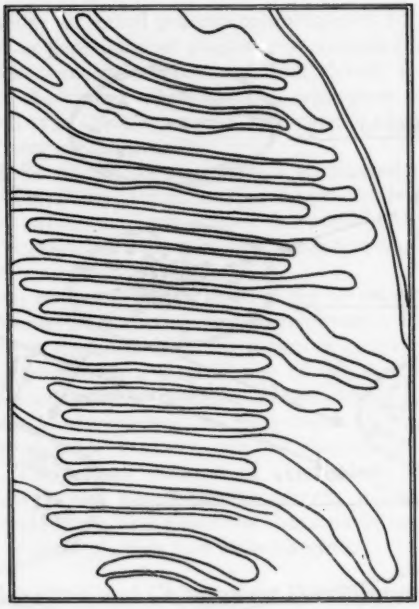

FIG. 2. Tracing of the lamellar structure in Fig. 3.

i.e. the aggregation of disks, has not been found, although the formation of large lamellar disks is possible. If etiolated plastids with prolamellar bodies are illuminated, the formation of primary layers can proceed, and grana are gradually developed.

The developmental sequence of chloroplasts, as outlined above, has been found in different species investigated, e.g., barley, tomato, tobacco, hops, and bean and agrees well with the findings of other investigators in the field (cf. 14, 7).

Most of the gene mutants discussed in this paper were induced in barley by radiations. Generally the *albina* mutants are blocked in the earliest stages of the development of the plastid structures. Two representatives are given in Fig. 1. *Albina*-12 can produce some vesicles by membrane invagination (Fig. 4) and seems to continue this process until the death of the seedling. However, the vesicles seem to be highly unstable since no accumulation in a prolamellar body has been found. A full-grown plastid of this type of mutant (*albina*-18) is reproduced in Fig. 5. Other *albina* mutants such as *albina*-20 accumulate vesicles in a prolamellar body. From a structural point of view young etiolated plastids represent phenocopies of such *albina* mutants. The full-grown *albina* plastids, although structurally related to mitochondria, are always considerably larger than mitochondria and may reach the size of a mature chloroplast. For comparison a mitochondrium can be viewed in

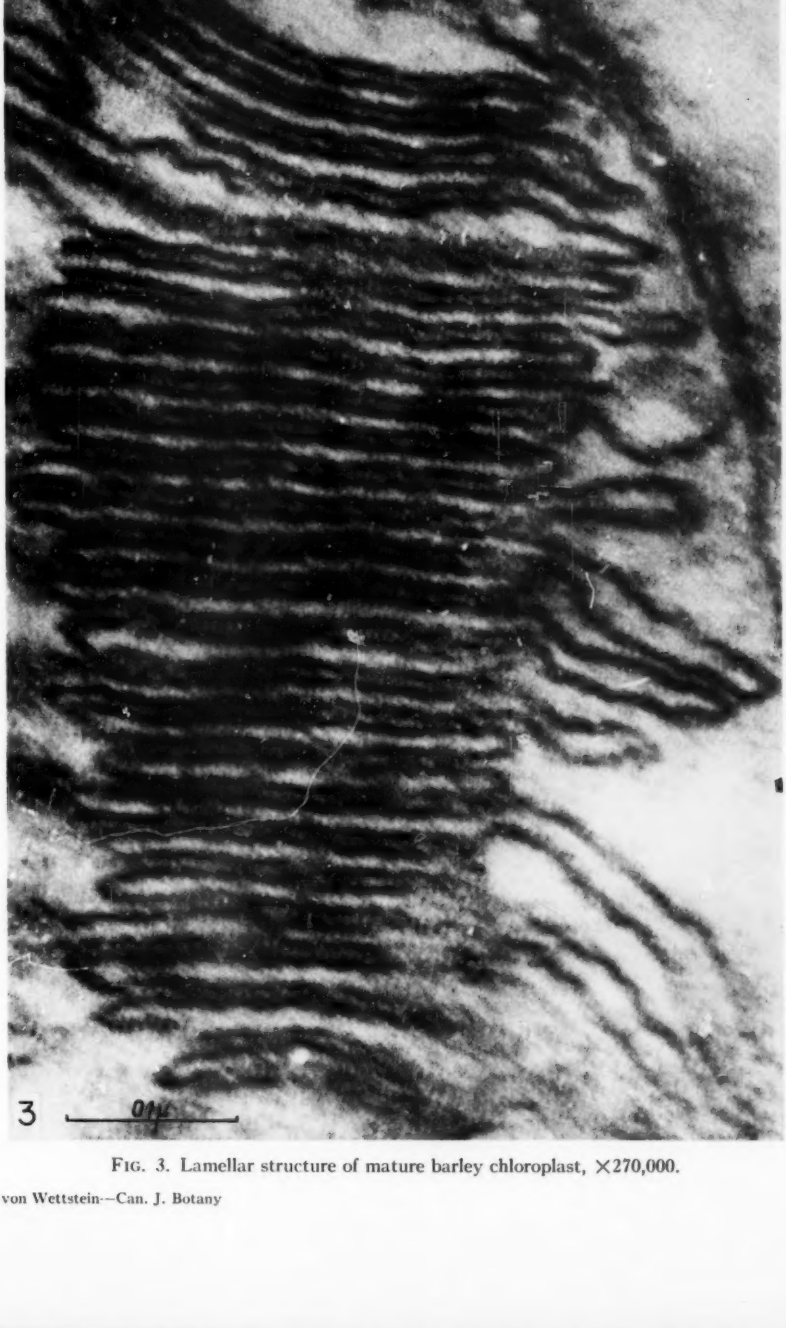

FIG. 3. Lamellar structure of mature barley chloroplast, ×270,000.

FIG. 4. A young proplastid or mitochondrium in *albina*-12 of barley, ×85,000.
FIG. 5. Plastid and mitochondrium in *albina*-18, ×24,000.

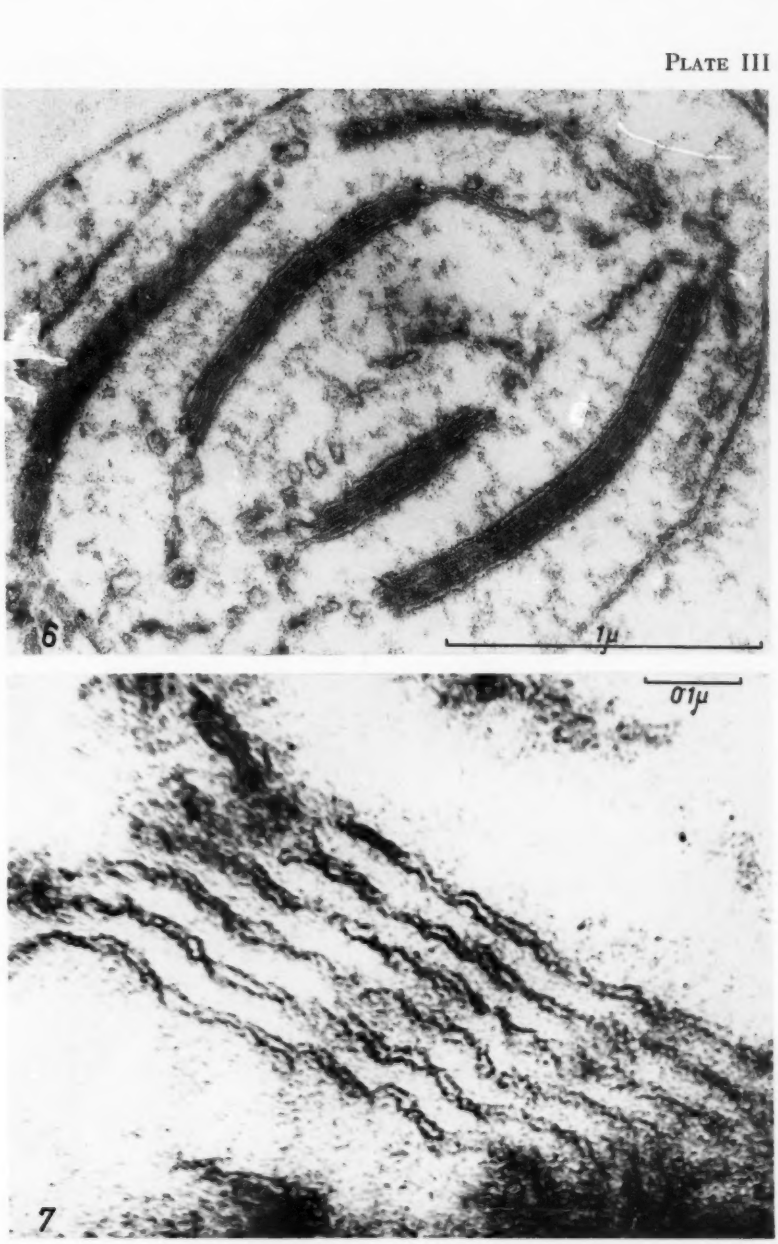

FIG. 6. Plastid of *xantha*-15, ×50,000.
FIG. 7. Lamellar structure in plastid of *xantha*-10, ×150,000.

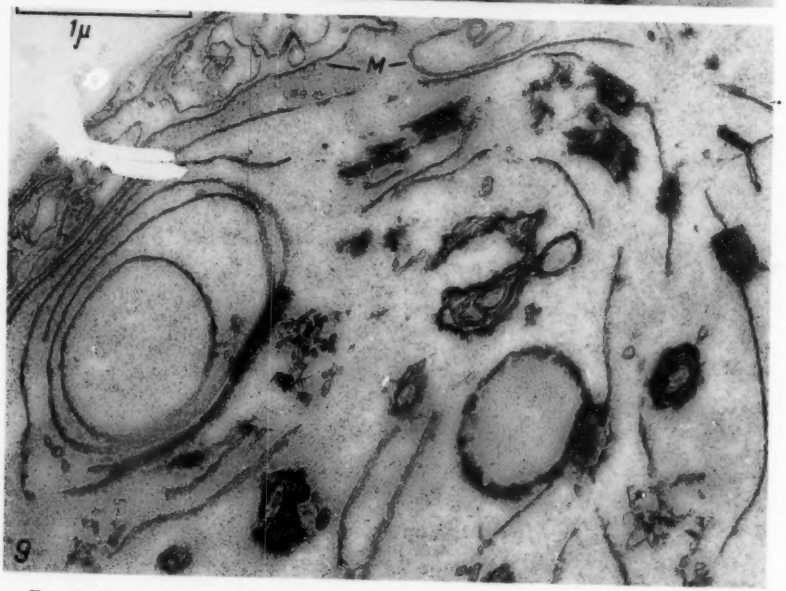

FIG. 8. Plastid from white area in variegated leaf of *Humulus japonicus*, ×36,000.
FIG. 9. Part of plastid and mitochondria from yellow sector in leaf of *striata*-4 in barley, ×27,000.

Fig. 5. As in maize (26), all *albina* mutants in barley which have been investigated in this respect can synthesize protochlorophyll and transform it into chlorophyll (37). Thus *albina* mutants are generally not blocked in chlorophyll synthesis.

A greater variety of blocks has been found in the yellow-colored *xantha* mutants. An interesting case is represented by *xantha*-10 (Fig. 1). This mutant cannot form protochlorophyll and probably has a block between protoporphyrin IX and protochlorophyll (37). It can produce vesicles and form large disks, which are widely spaced in a concentric arrangement within the plastid (Fig. 7). A phenocopy of this mutant can be produced by etiolating the plastids of barley for 3 weeks (35). Both in the mutant and in the phenocopy chlorophyll *a* is not formed, and the aggregation of the disks into grana structure does not take place. Perhaps the chlorophyll is a necessary constituent in the layer between the disks in the grana.

The mutant *xantha*-3 can synthesize chlorophyll and yellow pigments. These are, however, deposited into globuli, chromolipid droplets, and no lamellar system is formed. Large amounts of globuli are accumulated in the plastids of this mutant (32).

Xantha-15 shows another type of error (Fig. 6). The lamellar disks can be formed and even aggregated into large grana-like structures. The disks of the aggregates are much larger in diameter and the aggregation is less close than in normal grana, giving a thicker layer between the disks (38). Relatively large amounts of pigments are produced by this mutant. Either some constituent is missing or the surface of the lamellae have an aberrant structure preventing the proper aggregation of the disks. Like the other mutants discussed, this also is a lethal.

The later phase of the chloroplast development consists, as far as we can tell, in a multiplication and growth of the lamellar structure. The light-green *viridis* mutants show interference in this last part of plastid differentiation. Here quantitative differences are found in the number of lamellar disks. Many of these *viridis* mutants are more or less viable and are favorable material for a study of the quantitative relationships between photosynthetic capacity and amounts of chloroplast structure.

According to Röbbelen (21) *lutescens* mutants, that is mutants which are green in the early seedling stages and bleach later, show a secondary destruction of the chloroplast structure. The lamellar disks swell and the normal structural arrangement of the chloroplasts is destroyed.

This survey of different types of gene mutants cannot be considered exhaustive, and certainly other kinds of errors in the structural development of plastids will be discovered. However, I think present knowledge of the genic control of chloroplast development allows the conclusion that a mutational block can be found in every single step that leads to the formation of a mature chloroplast and its function.

Plastome and Plasmone Effects on the Structure of Chloroplasts

The status *albomaculatus* has been investigated in a white–green variegated form of *Nicotiana tabacum* (40) and the status *paralbomaculatus* in two *Oenothera* mutants (29). In all three cases normal chloroplasts are formed in the

cells carrying the plastome defect. The plastids are destroyed secondarily, whereby the lamellar disks swell and separate. Finally, the whole lamellar system is broken down and only a few vesicles remain in the stroma. The same behavior is displayed by the variegated form of *Humulus japonicus* (Fig. 8). There is thus no basic difference in the plastid phenotypes of these plastome and plasmone mutants. Various plastome mutants differ in the rate of destruction of the plastid structures as well as in its degree. Thus a yellow-green mutant in *Oenothera* does not proceed as far in the destruction of its lamellar system as a white mutant of this genus or the tobacco mutant. To a certain extent it is also possible to retard or prevent the destruction of the chloroplasts in such mutants by various environmental treatments (23, 25, 40), for instance by low light intensity and high temperature.

A different situation is encountered in two gene-dependent plastome mutations, *striata*-4, induced by X rays in barley, and a spontaneous one of this type, called "variegated", which was kindly supplied by Dr. Arnason, University of Saskatchewan (1). In both cases the phenotype of the plastids in the chlorophyll-deficient cells is that of a gene mutant. In Fig. 9 part of a plastid in a yellow sector of a leaf of *striata*-4 is shown. A great number of double membranes often in concentric arrangement are found, and also some abnormal grana-like packages occur. The details of the structural errors in this mutant are not yet understood, but it is noteworthy that simultaneously with this abnormal plastid structure the mitochondria are affected (M in Fig. 9). They are irregular in shape and size. The invaginations (cristae) of the mitochondrial membrane are few but strongly extended, undulated, or curled as compared with those in normal mitochondria from leaves of similar age (cf. Fig. 5). In some mitochondria all cristae are connected in a large double-membrane structure. The white–green variegated mutant of Dr. Arnason has typical *albina* plastids in the white sectors of the leaves. Also here the mitochondria show the abnormal structure found in *striata*-4.

Discussion

Gene-mutants affecting the development and function of chloroplasts are useful tools in two ways. Firstly we can learn from them about the formation of chloroplast structures and their function. Secondly, they are suitable systems for the study of gene-action.

It has been possible in microorganisms, such as *Neurospora* and bacteria, to clarify synthetic pathways by using biochemical mutants. Similar techniques have proved useful in the analysis of the biosynthesis of chloroplast pigments, chlorophylls, and carotenoids (8, 3). A way of determining a biochemical block is by finding the compound which as a consequence of the mutation can no longer be synthesized by the organism, and when supplied will overcome this block. That this technique can also be applied in higher plants has been shown by Langridge (10) in *Arabidopsis*, where a particular mutant has to be supplied with vitamin B_1 to grow normally. Recently it has become possible to overcome the blocks in differentiation of chloroplast structures in two barley mutants, *albina-7* and *xantha*-23, by supplying aspartic acid and leucine, respectively (31). Thus, we have the possibility

in chloroplast mutants of studying the effect of specific biochemical blocks in the formation of macromolecular structures. Eventually we may be able to locate processes such as protein synthesis in these structures by observing the changes happening when the block is removed.

Since various mutants in their blocked plastids often contain a structural organization comparable with developmental stages of normal chloroplasts we may inquire about the ability of the mutant plastids to perform processes and enzymatic activities involved in photosynthesis and thereby try to assign minimal structural requirements to the processes. We must bear in mind, however, that often the final stage of mutant plastids is not identical with that in which the structural development is arrested by the block.

We can see from the great variety of different gene-mutations affecting chloroplast development how strictly it is dependent on the information of the nuclear genes. The transfer of this information into the plastids is a fascinating problem.

As Correns (4, 5) has put it, the genes have to act on a "mechanism" in the cytoplasm, considered "as a chain of chemical processes, in which each process causes the next to follow". He thought that this chain of processes constitutes the basis of the extranuclear hereditary factors, and that the genes, via enzymes, interact in the development of the processes in the cytoplasm.

We may recall briefly what is known about the extranuclear factors affecting chloroplasts. From the work on *Oenothera* we know that two or even three different types of "plastome" factors can be contained in the zygote (27) and that these can segregate in the meristems. The segregation of plastome factors is strongly dependent on their rate of reproduction as shown by the competition experiments employing different plastomes and plastome mutations (22, 24). It has further been shown that different plastome factors causing plastid defects, when contained in the same plant, can give rise to intercellular complementation resulting in a normal phenotype of the plastids (28).

The extranuclear factors, and their behavior, can be recognized from the phenotype of the chloroplasts. Formally the results can be described by the mixing and segregation of different sorts of plastids. Baur (2), Renner (15, 16), and later for instance Schötz (24) and Stubbe (27) have concluded from their studies that the plastids themselves are the carriers of the extranuclear factors, and it would follow that the plastids or some part of them are autonomous self-duplicating organelles. Many cytological investigations on plastids have been undertaken to support the "Entmischungshypothese". The observation with the light microscope of differentiated so-called "mixed cells" with two or three light-microscopical distinguishable types of plastids has been taken as proof for this hypothesis (13, 27). As pointed out by Stubbe (27) and Schötz (24) the hypothesis requires proplastids in the meristems to differ. No case where two types of plastids can be distinguished in the meristem has been observed so far.

Quite apart from the unsolved question concerning what the observed differentiated mixed cells really are, the best available evidence on the development of chloroplasts does not support the independent cytological continuity of plastids (11, 12, 33, 34, 14, 7). They may very well arise from material

in the cytoplasm. In addition, mitochondria and early proplastids are indistinguishable from each other by present techniques. Division of plastids and mitochondria by fission in later stages of development does not allow conclusions about their origin in meristems.

The effect of the extranuclear factors in *Oenothera*, *Nicotiana*, and *Humulus* is observed late in plastid development as discussed above, and this is also shown by physiological studies in *Oenothera* (25). This seems to be rather general among the *albomaculatus* types, since many, if not all of them, can produce very young green leaves that are indistinguishable from normal leaves.

In the two gene-dependent or gene-induced plastome mutations described earlier both the plastids and mitochondria are affected, and the plastids seem to be blocked at relatively early stages. No conclusion about the location of these mutated extranuclear factors can be drawn at present.

Analysis of the reproduction of the chromosomal material and the action of genes in mutants has resulted in detailed hypotheses about the chain of events in the function of genes (cf. 30) which can be summarized briefly as follows: code in DNA ⟶ soluble RNA + protein synthesis on ribosomes ⟶ protein (enzymes) ⟶ products. There are regulators envisaged which limit the enzyme production, a phenomenon known as repression, and others which control enzyme activity, a phenomenon called feedback inhibition. In principle the extranuclear hereditary factors could reside in the ribosomes or constitute regulators, just to mention two possibilities. A determination of the biochemical and structural systems, which are controlled by extranuclear factors, is essential for a better understanding of these factors, their nature, their location, and their interaction with the nuclear genes.

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NOTES

PHYLOGENETIC SHIFTING OF ORGANS, TISSUES, AND PHASES IN PTERIDOPHYTES[1]

W. ZIMMERMANN

This note deals with a widespread and therefore important phenomenon in plants which has hitherto received little attention: the shifting of organs and tissues. Most morphologists are convinced that the position of organs and tissues is fixed both in phylogeny and in ontogeny. The position is considered to be unalterable during a change of form. According to the prevailing opinion, morphological alterations are mainly due to unequal growth or to the differentiation of organs and tissues without any temporal or spatial shifting.

In contrast to this opinion, I would like to bring to your attention some examples of phylogenetic shifting. Shifting means that the correlation of tissues and organs is altered in time or in space by alteration of genotype; organs and tissues are found at different places or different times relative to other organs and tissues.

A very obvious shifting of tissues is found in the phylogeny of the stele. Here the alteration can be followed very clearly, because the ancestor type and numerous fossil intermediate forms demonstrate the phylogeny. This phylogenetic shifting of the stele has already been demonstrated (Zimmermann 1954, 1959) and therefore it is only necessary to summarize:

(1) In the primitive stele (protostele, represented, for example, by *Rhynia*), a radial differentiation of tissues is found (Zimmermann 1959, Abb. 51). The visible anatomical radial gradient is determined by a physiologic gradient, because the cellular characters change step by step in the radial direction.

(2) The phylogenetic increase of meristeles or meristele-initials had started in the psilophytes by basipetal shifting of the stelar dichotomy (Fig. 1 and Zimmermann 1959, Abb. 51), to a lower point than the dichotomy of the exterior of the telome; therefore we find 2, instead of 1, meristeles in one telome. Or, by repeated equal or unequal shifting, we find 3, 4, 5, etc. traces or meristele-initials in a cross section.

(3) Another source of multiplication of meristeles is the overtopping of (simple or bifurcated) leaf traces. The beginning of the shifting is seen in *Botryopteris ramosa* Corsin with simple leaf-traces or in *Drepanophycus* according to Halle (Zimmermann 1959, Abb. 71). The increase in the number of meristeles to 7 or 8 (10) in *Lyginopteris* (Zimmermann 1959, Abb. 190) is an example of shifting of cauline and foliar traces also in seed plants. Renault described the basipetal shifting of foliar dichotomy in the cauline stele in the Permian *Sphenophyllum* quadrifidum (Zimmermann 1959, Abb. 104).

[1]Presented to the IXth International Botanical Congress, Montreal, 1959.

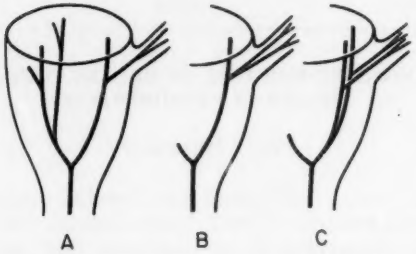

FIG. 1. Shifting of the meristeles from the leaf to the axis of a shoot: A, dichotomy still in the leaf; B, dichotomy shifted into the cortex of the axis; C, dichotomy shifted into the stele of the axis.

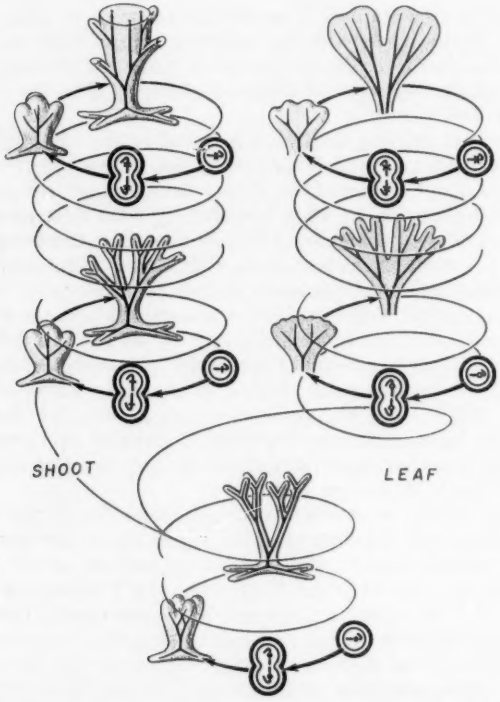

FIG. 2. Hologeny of the shifting in the shoot (left) and leaf (right) at the lower archisyntelomes.

PLATE I

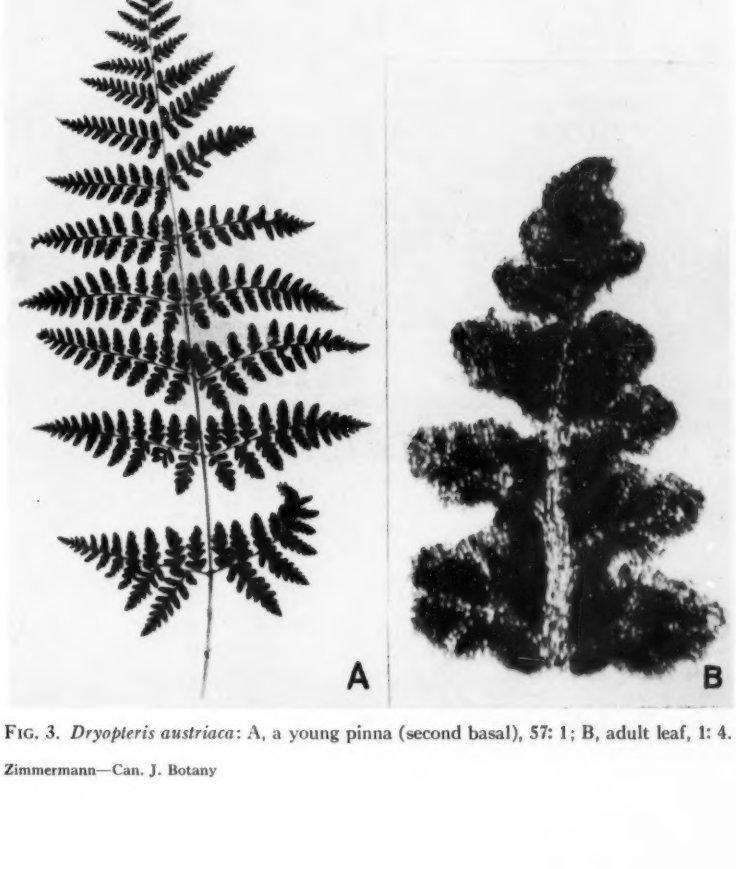

FIG. 3. *Dryopteris austriaca*: A, a young pinna (second basal), 57: 1; B, adult leaf, 1: 4.

The number of leaf-traces has increased in some Permian *Cordaites* for a leaf to 8 or in some Angiospermae to still more (Fig. 1).

(4) We can follow an ontogenetic shifting of traces in many seedlings, e.g. in the *Sphenophyta* and in some Angiospermae (Zimmermann 1959, Abb. 307). For instance, a single trace enters the stalk of *Pulsatilla* cotyledons, and an increasing number enter the stalks of the following leaves.

(5) The true development in these cases is the hologeny, which means that the phylogenetic shifting is caused by mutation of the genotype. The relative position of the stelar and parenchymatous tissues is altered in the growing points (Fig. 2).

A longitudinal phylogenetical shifting of organs exists, e.g., in pinnate leaves. The enormous number of fossil pteridophylla show clearly the course of phylogeny by shifting of determining factors in a longitudinal direction. The primitive archetype of the Pteridophyta (e.g., the Carboniferous *Rhodeopteridium*) has alternating, free, and catadromic pinnules. During the Paleozoic period nearly 100% of the leaves are catadromically organized.

In the majority of the living ferns, the positions of the pinnae and pinnules have phylogenetically shifted, e.g., the most basal pinna is shifted in the opposite position. Moreover, the structure of the pinnae is sometimes purely anadromic, i.e., in the Aspleniacae, *Cystopteris*, *Davallia*, etc. The Dryopteridaceae (including *Athyrium filix femina*) are more complicated. At the base of a pinna the position of the pinnules is catadromic and higher up anadromous; so we have a point with opposite position of pinnules at the 3–7 pair of pinnules (Zimmermann 1959, Abb. 167).

While a shifting in the opposite position reduces the internodes to zero, a further shifting leads to something like internodes of negative value. This indicates that the shifting is not at all a reduction process in the rachis but a true shifting of determining factors. This is also proved by the fact that the pinnae and pinnules are, in the embryonic stage, in the same relative position in which we found them in the adult leaves (Fig. 3).

The shifting leads, e.g., in *Dryopteris austriaca*, to an anadromous position: 2 pinnules on the lower side correspond in some races to 3 pinnules on the upper side (Fig. 3A). The same position of pinnules is to be seen in embryonic leaves (Fig. 3B).

The modern type of pteridophylla begins to develop in the Mesozoic period by shifting of pinnae and pinnules according to the investigations of A. M. Schneider, who has worked with me. About 30% of the pinnae at the leaf-base are shifted in the opposite position, and in 24% of Mesozoic leaves of *Cladophlebis* and *Todites* we find the *Athyrium*-type with basal catadromous and apical anadromous pinnules.

Similar cases of phylogenetic shifting of organs in the direction of the longitudinal polar gradient of axes which characterizes all cormophytes are found by a comparative study in embryos. Here, too, the shifting of organs is the consequence of shifting of organ-determining factors. Even the determination of stem and root pole (the primitive spindle of Bower) is changed during phylogeny. Some embryos are endoscopic and others are exoscopic (e.g. Zimmermann 1959, Abb. 57).

Sometimes unequal shifting of different phylogenetic elementary processes alters the morphology and anatomy. I will give some examples for the Angiospermae that show it well.

In my genetical investigations on *Pulsatilla*, I succeeded in obtaining quite new organs by combination of two mutants: (1) a mutant with acropetal-shifted laciniating factor and therefore with laciniated outer tepals, and (2) a mutant with acropetal shifting of a tepal-determining factor and therefore numerous (8–11) tepals. By this combination I obtained plants with a supernumerary outer laciniate whorl of organs and two inner unaltered whorls of the normal flower (Fig. 4D).

Also a basipetal shifting of the border of androeceum-determining factors creates a new organ. In place of the inner petals, an organ which combines the characteristics of the androeceum and the tepals (Fig. 4C) is found; a broadened petiole resembles the tepals, and the apical part of this new organ corresponds more to stamens or the normal nectary.

We can conclude that this manner of shifting of the organ-determining factors and their new combination is the path to new organs in the natural phylogeny.

Quite new forms arise too in the reverse case, when elementary processes which are normally combined are separated by unequal shifting. Experimentally changed twigs of *Euphorbia cyparissias* show this shifting. A poorly nourished plant (the soil consisted of 4 parts sand and 1 part mold) and a well-nourished plant (the soil consisted only of mold) show, in Fig. 5A and C, the different combination of long and short internodes with the shifted position of the border between vegetative and floral parts.

The shifting in time, for instance the shifting of the two cardinal points of the alternation of the nuclear phases, will now be treated briefly. The reduction-division can be shifted from the zygote to the agametangia, which (see Zimmermann 1930) has led to the isomorphic alternation of generations. Or, by shifting of reduction-division to the gametangia, the type of prevailing diplonts has originated (Fig. 6A, D, B).

But also the other cardinal point, the fusion of nuclei, can be shifted to other stages of development. In the case of restitution nuclei (e.g., in *Dryopteris palaeacea*), the fusion of nuclei is shifted from the gametophytic generation into the sporogenous tissue. Thus the gametophyte and the sporophyte are haploid and follow each other without alternation of nuclear phases and yet at once, after the fusion of nuclei in the sporogenous tissue, a reduction-division will produce normal haploid spores (Fig. 6C).

A similar shifting in time is the neoteny, which is the shifting of the production of reproductive organs on a vegetative juvenile stage. The reduction processes of gametophytes in Laminariales or in Pteridophyta, or the reduction of the sporophytes in *Cutleria* and in Bryophyta, are examples of neoteny or shifting of the reproductive phase (Fig. 6E).

The end of the reduction series in the gametophyte (which is a progressive neoteny) is shown by the gymnosperm- and angiosperm-series pointed out by Hofmeister (Zimmermann 1959, Abb. 264). In the majority of gymnosperms (for instance, in *Ephedra*) the female gametangia are formed when the sporophyte is divided still by cell divisions. As a further stage of neoteny in

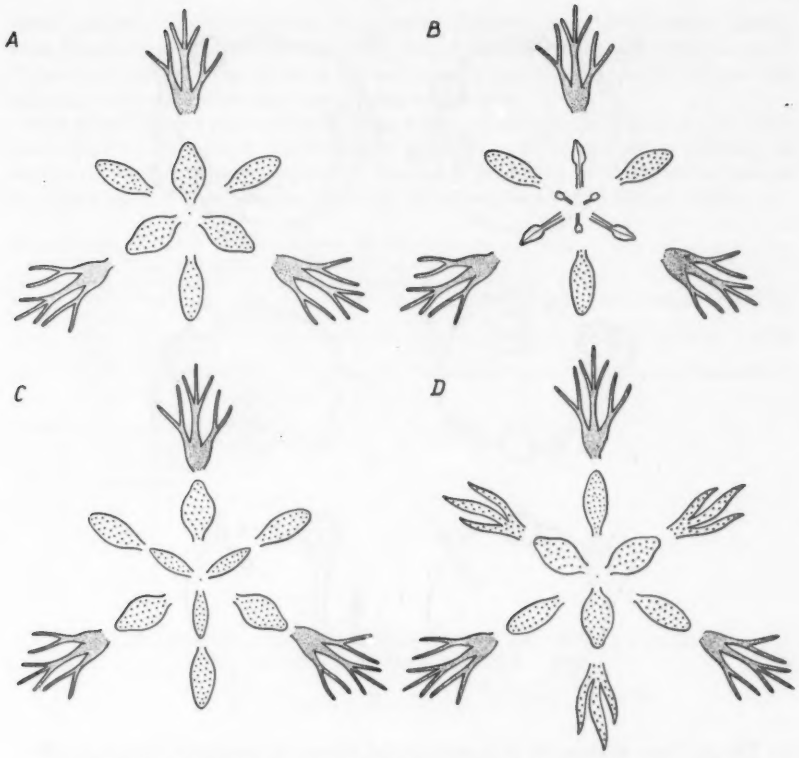

FIG. 4. *Pulsatilla* flower schemes: A, normal flower; B, tritepal flower; C, supernumerous tepals; D, new outer (laciniate) whorl of perianth. Finely dotted = green colored; sparsely dotted = violet colored.

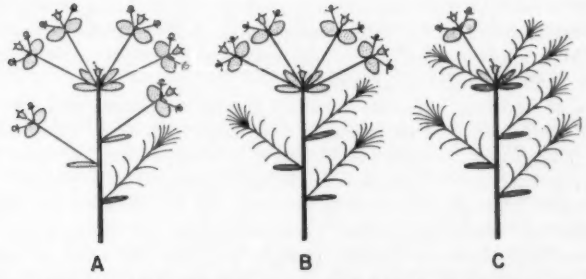

FIG. 5. *Euphorbia cyparissias:* A, basipetal shifting of floral determination; B, "normal" type; C, acropetal shifting of vegetative determination. Lined = green colored; dotted = yellowish green colored.

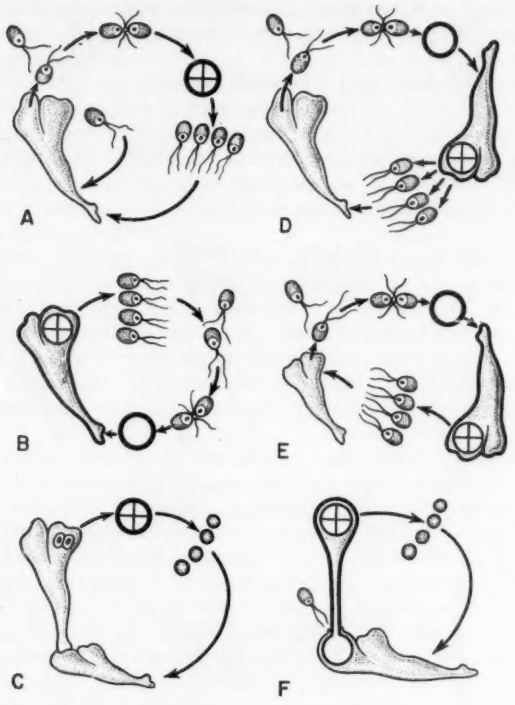

FIG. 6. Types of alteration of generations and phases: A, haploid; B, diploid; C, apogamy (restitution nucleus); D, diploid–haploid (isomorphic); E, diploid–haploid (heteromorphic); F, diploid–haploid (sporophyte reduction).

Welwitschia, the egg is formed before the formation of cell walls in the sporophyte. And before cell walls are formed between the four megaspores (produced by the reduction-division), even before any differentiation can take place between these spore-nuclei, the whole megaspore mother cell develops to a tetrasporic embryosac, e.g. in *Gnetum*.

As cases of neoteny, one can consider the development of herbs or of the number of ovules as is to be found in the Ranunculaceae, where (as the last link) the total mass of embryonic tissue is determined to form one ovule before a differentiation of single initials of ovules can take place.

An extreme example of neoteny is seen in the flowers of *Balanophora* which, together with the other representatives of Santalales, including Balanophoraceae, form a nearly complete series of neoteny of reduced flowers. The last link in this series is an archegoniumlike organ, which (by neoteny) develops from the growing point of a female flower, and which in its interior contains only the embryo sac, surrounded by a thin layer, which

often consists of only one layer of cells (Zimmermann 1959, Abb. 285b). This means also that the determining factor for initiating the embryo sac is shifted into the growing point at a very early stage. There, among only a few cells the embryo-sac mother cell begins to develop.

The great importance of the diverse forms of shifting is shown by the fact that there is no recent tracheophyte without well-recognizable shifting of organs or tissues. Many frequently discussed problems are only to be solved by following this phylogenetic shifting (Zimmermann and Hanser 1962).

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A SALKOWSKI-POSITIVE DERIVATIVE OF FERULIC ACID FROM WHEAT LEAVES

B. I. SAHAI AND MICHAEL SHAW

During an investigation of growth substances in rusted and uninfected wheat leaves, a spot which gave a crimson color with the Salkowski reagent (after 45 minutes at room temperature) was observed at an R_f value of 0.5–0.6 on chromatograms of the neutral ether-soluble fraction developed in isopropanol:water (10:1) in an atmosphere equilibrated with isopropanol:water:ammonia (10:1:1). A faint yellowish color giving a blue fluorescence under ultraviolet light was observed at this R_f on unsprayed chromatograms. The substance had no effect on the extension growth of *Avena* coleoptile sections.

In order to determine the nature of the unknown substance, 4 kg of Little Club wheat leaves were harvested, frozen in dry ice, ground to a powder in the cold, and extracted overnight with absolute alcohol at $-10°$ C. The alcohol was removed *in vacuo* at 35° C. The aqueous residue was filtered, brought to pH 8.5 with 5% sodium carbonate, and extracted three times with peroxide-free ether (7). The ether extract was then washed with water, dried over anhydrous sodium sulphate overnight in the refrigerator, concentrated, and chromatographed on Whatman No. 1 paper. The appropriate section of the

chromatogram was located by spraying a guide strip with the Salkowski reagent and by examination under ultraviolet light. It was then eluted with ether and the eluate rechromatographed twice in the isopropanol solvent and evaporated to dryness. The residue was used for determination of ultraviolet and infrared spectra. These were similar to, but in neither case identical with, spectra of ferulic acid and methyl ferulate. The unknown substance gave pink colors with diazotized-*p*-nitroaniline and diazotized sulphanilic acid (1). It did not react with Ehrlich's reagent (7). Its R_f values in toluene:acetic acid:water (4:1:5) and butanol:acetic acid:water (4:1:5) were 0.05 and 0.88 respectively.

Methyl ferulate and 4-methoxymethyl ferulate gave pink colors with the Salkowski reagent, but their R_f values in isopropanol:water and in toluene:acetic acid:water were different from those of the unknown.

After hydrolysis with 2% HCl at 100° C for 1 hour, in addition to unhydrolyzed residue, the unknown substance gave rise to two other distinct spots on chromatograms developed in either isopropanol:water or toluene:acetic acid:water. Both of these new spots appeared blue under ultraviolet light. Their color reactions with diazotized *p*-nitroaniline and diazotized sulphanilic acid were characteristic of paracoumaric and ferulic acids (1). In all three solvent systems mentioned above, their chromatographic behavior was identical with that of synthetic paracoumaric and ferulic acids. Ferulic, caffeic, sinapic, and chlorogenic acids react with the Salkowski reagent to give a crimson color 1 hour after spraying at room temperature. Paracoumaric acid does not react with this reagent. All the above acids gave a blue-purple color with Ehrlich's reagent after 1 hour at room temperature. Booth (3) has drawn attention to the fact that Ehrlich's reagent is not specific for indoles and reacts with sugars.

The results presented above suggest either that the unknown compound is a mixture of a derivative of ferulic acid and a derivative of paracoumaric acid, both of which behave identically in the chromatographic solvents employed, or that the unknown compound is a derivative of both ferulic and paracoumaric acids. Esters of ferulic and paracoumaric acids have been reported in a variety of plant materials (4, 6, 8) and *p*-coumaryl quinic acid occurs in apples (9).

One object of this note is to draw attention to the possibility that since naturally occurring oxycinnamic acids and their derivatives react slowly with the Salkowski and Ehrlich's reagents, they may be mistaken for indole compounds unless identifications are based on other more specific criteria. In work on growth substances this is all too often not the case (see reviews by Bentley (2) and Gruen (5)).

This work was carried out with financial assistance from the National Research Council of Canada.

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THE RELATION OF BARK MOISTURE TO THE DEVELOPMENT OF CANKER DISEASES CAUSED BY NATIVE, FACULTATIVE PARASITES

VI. PATHOGENICITY STUDIES OF HYPOXYLON PRUINATUM (KLOTZSCH) CKE., AND SEPTORIA MUSIVA PK. ON SPECIES OF ACER, POPULUS, AND SALIX[1]

JOHN E. BIER

Introduction

Forest pathologists in many countries are considering the serious problems that may arise through the movement of internationally dangerous diseases from one country to another. As a preliminary step in facilitating this program, Riker (9) as a member of the working group on Foreign Tree Diseases in Section 24 (Protection) of the International Union of Forest Research Organizations has consulted with forest authorities in 16 countries.

Riker (9) lists *Septoria* canker, and Krstic (7) and Imazeki (6) consider both *Hypoxylon* and *Septoria* cankers, as North American tree diseases potentially dangerous to the forests of foreign countries.

The results of previous studies (1, 2, 3, 4, 5) in the field and in the laboratory have demonstrated a close correlation between the development of bark cankers caused by facultative parasites and the relative turgidity level of young, living bark. The uniformity in the results of investigations with three pathogens on different genera and species of trees introduced the possibility that international co-operation for the screening of some canker diseases caused by facultative parasites may be accomplished by pathogenicity tests on dormant cuttings of the hosts, to establish the threshold levels of bark turgor which indicate disease susceptibility. After the threshold level has been deter-

[1]Contribution from the Department of Biology and Botany, and the Faculty of Forestry (Research Paper No. 39), University of British Columbia, Vancouver 8, B.C.

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mined for a host and pathogen, it may be possible to relate this value to the field behavior of the trees as it occurs in different countries. The assignment of disease vulnerability ratings to different stands and plantations of the host may be feasible.

To obtain further information in support of these hypotheses pathogenicity studies of *Hypoxylon pruinatum* (Klotzsch) Cke. and *Septoria musiva* Pk.[2] were made on dormant cuttings harvested from field trees of the following species in the University Forest, Vancouver: *Acer macrophyllum* Pursh. (broadleaf maple), *Populus tremuloides* Michx. (trembling aspen), *P. trichocarpa* Torrey and Gray (black cottonwood), *Salix* sp. (probably *S. scouleriana* Barratt).

It is of interest that neither *Hypoxylon* nor *Septoria* cankers have been observed in the Vancouver area.

Finally, it was considered that the results of these studies might provide a contribution to Part 6 of Riker's (9) suggested program on internationally dangerous diseases, which refers to the acquisition of badly needed information about the host, pathogen, and disease development.

Methods

The methods of determining the relative turgidity of bark and preparing the wound inoculations were the same as those described in the studies of *Cryptodiaporthe* canker (1).

During the dormant season of 1960–61 cuttings were harvested from the 1960 growth of the different trees and measurements were made of their bark moisture content. All cuttings were made to a uniform size, namely, 25 cm long with a mid-diameter of 1 cm, and were weighed to 0.1 g accuracy. After the ends were sealed with paraffin, one inoculation and a control were made on each cutting and, after treatment, one-half of each sample of 12 cuttings was placed in a plastic bag and incubated in a cabinet with a temperature ranging from 4° to 7° C. The remainder of each sample was placed in a moist chamber at laboratory temperature.

After canker growth was observed, the diseased cuttings were weighed again and a second series of bark moisture measurements was made. Fresh cuts were made at the basal ends of the cuttings before placing them in beakers containing water to a depth of approximately 2 cm. The material was then stored temporarily in a refrigerator at a temperature of approximately 5° C. This

[2]Mr. B. W. Dance, Forest Pathology Laboratory, Southern Research Station, Maple, Ontario, kindly provided the multispore cultures of *H. pruinatum* and *S. musiva* which served as the sources of inoculum in this study.

FIGS. 1–10, 13. Upper wounds controls, lower wounds inoculations.

FIGS. 1–8. Figure legends as follows: Inoc. = date of inoculation, Dis. = date canker growth was observed, Water 5° C = period of storage in water at 5° C, Growth = period of planting.

FIGS. 1–4. *Hypoxylon* canker on *A. macrophyllum*, *P. tremuloides*, *P. trichocarpa*, and *Salix* sp. respectively. FIGS. 5–8. *Septoria* canker on *A. macrophyllum*, *P. tremuloides*, *P. trichocarpa*, and *Salix* sp., respectively.

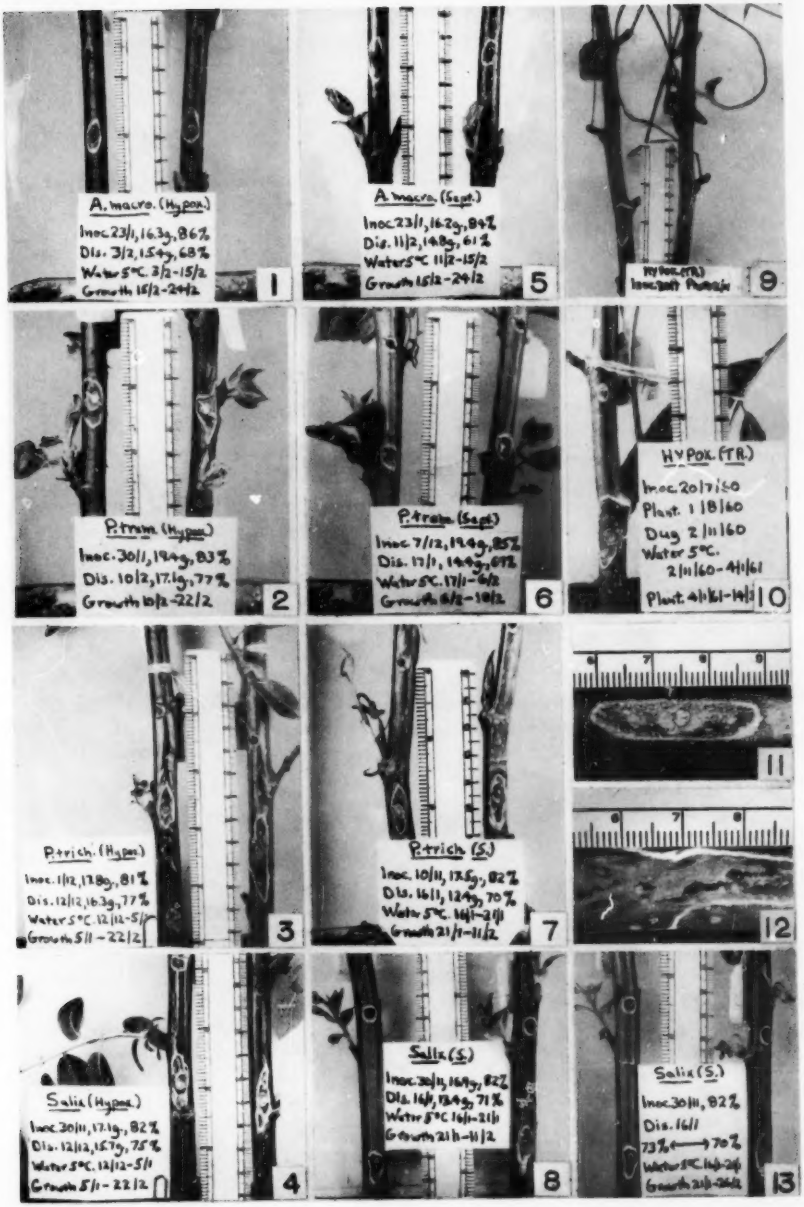
FIG. 9. *Hypoxylon* canker on *P. trichocarpa*, 105 days after inoculation.

FIG. 10. As Fig. 9, 237 days after inoculation.

FIG. 11. Pycnidia of *S. musiva* on inoculated cutting of *P. trichocarpa*.

FIG. 12. Conidial stage of *H. pruinatum* on inoculated cutting of *P. trichocarpa*.

FIG. 13. *Septoria* canker on *Salix* sp. Compare with right-hand cutting in Fig. 8, and note canker extension which occurred subsequent to the production of the new shoots.



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treatment provided a method for replenishment of water in the partially desiccated cuttings and gave more assurance that the material had completed the period of "physiological" dormancy as described by Richardson (8). After the chilling period the cuttings were planted in an equal mixture of garden soil and vermiculite at laboratory temperature.

Results

It was evident that both methods of incubation were satisfactory in this work. Canker development occurred earlier in the material at room temperature, which may have been due to more rapid moisture loss and the more favorable temperature for the growth of the pathogens. In some instances conidia of the pathogens developed on the cankered areas (Figs. 11 and 12), and no difficulty was encountered in reisolating the pathogens from diseased bark. Most of the inoculated cuttings produced new shoots after planting which established their viability at the time of canker growth (Figs. 1 to 8).

The results of the experiments demonstrated that *H. pruinatum* and *S. musiva* were causal agents for canker diseases on *A. macrophyllum*, *P. tremuloides*, *P. trichocarpa*, and *Salix* sp. when the bark tissues of the hosts were conditioned for disease development. Summaries of the inoculation, total cutting weight, and bark moisture studies are presented in Table I and in Figs. 1 to 8.

In Table I it is evident that the average relative turgidity of the dormant bark of all host species was above 80% at the times of harvest. In each host–pathogen association canker development occurred only when the relative turgidity of the bark was below the 80% level (Table I).

In the cuttings of each host the development of *Hypoxylon* canker occurred within shorter periods of incubation and with smaller losses in total weight and bark moisture than that of *S. musiva* (Table I). Indeed, an examination of the average percentage losses in total weight and bark moisture level at the time of canker development shows a degree of uniformity in the parasitic behavior of the two pathogens, particularly on the poplars and willow (Table I). From these experiments it appeared that *H. pruinatum* was a more virulent pathogen than *S. musiva*, since it attacked more vigorous bark as expressed by the higher values of bark moisture. The threshold levels of bark moisture for canker growth in the poplars and willow, expressed as relative turgidity percentages, were approximately 75 to 77% for *H. pruinatum*, and 69 to 71% for *S. musiva* (Table I).

The difference in the degree of virulence of the two pathogens was also indicated by comparing the average weight losses in the standard-sized cuttings (Table I). The losses in total weight were considerably greater at the time of canker development by *S. musiva* than by *H. pruinatum*. These results provided additional evidence to that reported previously for *P.* 'robusta' (5), when it was suggested that the average total weight of uniform-sized cuttings from the previous year's growth may serve as a practical indicator for degree of host vigor as expressed by its vulnerability to some canker diseases.

From Table I it is apparent that the total weight and bark moisture losses at the time of canker development in *A. macrophyllum* were quite different from those recorded for the other host trees. The bark of the maple cuttings

TABLE I

Summary of inoculation, total weight (cutting 25 cm long with mid-diameter of 1 cm), and bark moisture studies on host material allowed to dry gradually in a dormant condition

Tree species	Pathogen	Total no. of successful inoculations*	Incubation period before canker development (days)	Av. values at time of harvest		Av. values at time of canker growth		Av. percentage loss in:	
				Av. total wt., g	Av. rel. turg., %	Av. total wt., g	Av. rel. turg., %	Total weight	Rel. turg.
A. macrophyllum	*Hypox.*	21	11	16.3	86	15.4	68	6	20
	Sept.	12	19	16.2	84	14.8	61	9	27
P. tremuloides	*Hypox.*	24	11	19.4	83	17.1	77	12	7
	Sept.	14	41	19.4	85	14.4	69	26	19
P. trichocarpa	*Hypox.*	36	11	17.8	81	16.3	77	9	5
	Sept.	27	67	17.5	82	12.4	70	29	15
Salix sp.	*Hypox.*	36	12	17.1	82	15.7	75	8	9
	Sept.	34	47	16.9	82	13.4	71	21	13

* Inoculation failures consisted of diseased cuttings which failed to produce shoots after planting.

was extremely thin with the result that it lost water very rapidly, accompanied by heavy lenticel and nodal infection by *Cephalothecium roseum* Cda.

Canker growth was arrested in each host–pathogen association when water was supplied to viable, dormant cuttings stored at temperatures favorable to the growth of the pathogens. In such instances the relative turgidity of the bark was increased to above the 80% level within a period of 48 hours (Figs. 1 to 8).

The provision of a continuous water supply was not successful in arresting the development of *Septoria* canker in approximately 30% of inoculated cuttings. In these instances canker growth continued and was accompanied by a general deterioration of the bark tissues over the entire surface of the cuttings. It was evident that this material had been dried to a level that prevented later recovery. In some cuttings infected by *S. musiva*, canker growth did not continue until after the material had been planted for a period of approximately one month. These cuttings produced new shoots but were unable to develop basal calluses and adequate root systems (Fig. 13). Since the threshold level for canker development by *S. musiva* occurred in bark with a relative turgidity of approximately 70%, it was possible that some cuttings were seriously injured as the result of this amount of desiccation.

In many of the *Hypoxylon* inoculations some canker growth occurred after the cuttings were planted (Figs. 3 and 4). Apparently this attack was related to the period of moisture stress which occurred in the cuttings during the interval between the initiation of shoot growth and the formation of the new root systems. Most of this canker growth was along the stems rather than around their circumferences (Figs. 3 and 4). Canker growth has not been observed in diseased cuttings with well-developed root systems.

It may be of interest to outline the results of inoculation experiments of *H. pruinatum* on *P. trichocarpa* which were initiated during the summer of 1960. On July 20, cuttings were harvested from shoots that had developed in 1959 (av. rel. turg. of the bark was 93%). The cuttings were inoculated and canker growth was evident after 12 days of incubation in a moist chamber at room temperature (av. rel. turg. of the bark was 76%). The diseased cuttings were then planted in vermiculite and allowed to grow until the completion of natural defoliation on Nov. 2. Limited canker development occurred after the cuttings were planted (Fig. 9). On Nov. 2 the cuttings were removed from the vermiculite and the below-ground portions placed in a beaker of water (av. rel. turg. of the bark was 84%). From Nov. 2 to Jan. 4, 1961, the material was stored in this manner in a refrigerator at a temperature of approximately 7° C. Although *H. pruinatum* is able to grow at this temperature, canker development did not occur during this period of storage (av. rel. turg. of the bark was 83%). On Jan. 4, the cuttings were replanted in a mixture of garden soil and vermiculite. New shoots and roots were produced by a number of these cuttings without further disease spread (Fig. 10). Despite the severe treatment given to these cuttings during growth, and in dormancy, canker growth was prevented by maintaining the relative turgidity of the bark above the 80% level.

Discussion and Summary

Neither *Hypoxylon* nor *Septoria* canker has been found in the field in the

Vancouver area. However, by subjecting cuttings to a period of drying, the pathogens for these diseases attacked *A. macrophyllum*, *P. tremuloides*, *P. trichocarpa*, and *Salix* sp. harvested from the University area. Experiments have shown that canker growth by these pathogens could be arrested by maintaining the relative turgidity of the host bark above the 80% level. These results applied to host material in growth and in dormancy. As in the earlier studies (1, 2, 3, 4, 5) bark moisture measurements provided useful indices for the level of host resistance to these diseases.

During 1960 and 1961 numerous measurements have been made to determine the field levels of bark moisture in the hosts mentioned. In all instances, whether in growth or in dormancy, the average relative turgidity of the 1-year-old bark has been above the 80% level indicating resistance to these diseases. Therefore, it is possible that within this area the degree of host vigor as expressed by the bark moisture level is seldom below the 80% indicating canker susceptibility.

The threshold levels of bark moisture for susceptibility to *Hypoxylon* canker were very similar in *P. tremuloides*, *P. trichocarpa*, and *Salix* sp., the average relative turgidities varying from 75 to 77%. This also applied to *Septoria* canker; only in these instances the threshold levels for susceptibility were lowered to average relative turgidities between 69 and 71%. From these results it was concluded that *H. pruinatum* was the most virulent pathogen, since it attacked more vigorous bark as indicated by the higher values in bark moisture. Therefore, under field conditions it may be expected that *Hypoxylon* canker would occur in more vigorous trees or stands of these hosts than *Septoria* canker. Indeed, the relative incidence of these fungi as canker pathogens on *P. tremuloides* in eastern and central North America would support this conclusion. If *Septoria* canker has not been found on *P. tremuloides* it may be that under field conditions the degree of vigor of this host has seldom, if ever, been reduced to the threshold level for susceptibility, during periods when the atmospheric temperature was favorable to the growth of the pathogen.

In the standard-sized cuttings of *P. tremuloides*, *P. trichocarpa*, and *Salix* sp., the losses in total weight were considerably greater at the time of canker development by *S. musiva* than by *H. pruinatum*. Therefore, the average weight of samples of dormant cuttings harvested from the previous season's growth and cut to a uniform size may indicate the vigor level of the trees and their relative vulnerability to these canker diseases.

The information presented in the preceding paragraphs mentions two methods which may be of value in an international program for the screening of some canker diseases caused by facultative parasites. Pathogenicity tests on dormant cutting stock harvested from the previous year's growth may be adequate for the establishment of threshold levels which indicate host susceptibility, and which are based upon bark turgor level and (or) average total weight of uniform-sized cuttings. These threshold levels could be related to the field behavior of the host trees in different countries.

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